ARCHIVES OF PATHOLOGY

Volume 44
1947

CONTENTS OF VOLUME 44

iii

OCTOBER—*Continued*

NOVEMBER 1947. NUMBER 5

DECEMBER 1947. NUMBER 6

STUDIES ON THE PATHOGENESIS OF RHEUMATIC FEVER

I. Experimental Production of Autoantibodies to Heart, Skeletal Muscle and Connective Tissue

PHILIP A. CAVELTI, M.D.

SAN FRANCISCO

IN 1934 Burky[1] showed that rabbits receiving injections of toxic filtrates of Staphylococcus aureus which had been grown on broth prepared from lens substance became sensitized to plain lens substance, as evidenced by cutaneous reactions. He was able to effect similar autosensitization to rabbit muscle extract by using as an immunizing agent a toxin prepared by growing the organisms on a medium containing rabbit muscle. Staphylococcus toxin, therefore, has the ability to confer antigenicity on substances of tissues of animals, with consequent formation of isoantibodies to these tissue substances within animals of the same species.

Using kidney in connection with staphylococcus toxin, Schwentker and Comploier[2] obtained complement-fixing antibodies to plain rabbit kidney. Streptococcus toxin was found by them to be only slightly effective in conferring antigenicity on rabbit kidney.

Precipitins to extracts of rabbit skin were produced by Hecht, Sulzberger and Weil[3] in rabbits by immunizing these animals with a combination of rabbit skin emulsion and staphylococcus toxin.

In our previous experiments[4] we have shown that autoantibodies to kidney can be produced both in rabbits and in rats by injecting renal material in combination with streptococcic substances, especially whole killed group A beta hemolytic streptococci. The autoantibodies were demonstrable in vitro (for rabbits, often in high titers) by means of the collodion particle technic. Further, in rats, as a result of the in vivo reaction of the autoantibodies with the kidney in situ, acute and chronic

1. Burky, E. L.: J. Allergy 5:466, 1934.

2. Schwentker, F. F., and Comploier, F. C.: J. Exper. Med. 70:223, 1939.

3. Hecht, R.; Sulzberger, M. B., and Weil, H.: J. Exper. Med. 78:59, 1943.

✓4. Cavelti, P. A., and Cavelti, E. S.: Arch. Path. 39:148, 1945; 40:158 and 163, 1945.

glomerulonephritis was produced. These results lend support to the concept that human glomerulonephritis also may be due to specific auto-antibodies to kidney formed as a response to an antigen arising from a combination of streptococcic substance with renal material during the streptococcic infection which almost invariably precedes human glomerulonephritis by at least a few days, usually one to three weeks.

In the experiments to be reported here, the question was investigated whether streptococci, in an analogous manner, are able to render substances from certain other tissues antigenic, thus leading to the production of autoantibodies to these tissue substances. It was further studied whether any such autoantibodies formed are capable of damaging the corresponding tissues by their reaction with the antigen in situ. From a clinical point of view, rheumatic fever could conceivably be due to such mechanisms, as suggested by the relation of this disease to a preceding streptococcic infection. As these experiments were carried out with a view to the genesis of rheumatic fever, the tissues chiefly employed in this study were heart, connective tissue and muscle, the structures which are predominantly affected by the rheumatic lesions.

MATERIALS AND METHODS

Preparation of Tissue Materials.—For most of the experiments which were done on rats, tissues were employed which had previously been washed free of blood by perfusion of the whole animal with isotonic solution of sodium chloride under sterile conditions. With the rat under ether anesthesia, a cannula was introduced into the left ventricle immediately after the chest was opened, and, with a clip of the scissors, an opening was made in the right auricle. Two minutes usually were sufficient for perfusion of the heart, but when other tissues were to be used, perfusion was continued for about five to ten minutes.

Heart and kidneys were removed aseptically. To obtain skeletal muscle and connective tissue some of the rats then were freed of all internal organs and of the entire skin and the head under as clean conditions as possible but not aseptically. The carcass was rinsed under running tap water and cut into pieces. The skin was fixed with the inside up on a wooden board and the subcutaneous fat and connective tissue were collected.

All the tissues were kept frozen, either in an electric freezer at —18 C. or in the carbon dioxide ice box at —76 C., until used. In the latter case, tightly stoppered containers were used to prevent carbon dioxide vapors from coming in contact with the material.

Preparation of Tissue Emulsions.—In some experiments in which relatively small amounts of tissue were involved, the tissues were ground in a mortar with sand, saline solution being added to make a 20 per cent emulsion by weight. This method, however, was found too strenuous for larger amounts, especially for material rich in connective tissue. For larger amounts the following methods were employed:

Heart and Soft Tissues: The tissues were minced and emulsified in a Waring blender of appropriate size. This was usually effected in one-half to three minutes, care being taken to avoid overheating. The suspension was then centrifuged for a short time at low speed to remove coarse materials, which consisted chiefly of

lumps of connective tissue and, when desirable—as in the case of heart—were ground separately in a mortar and added to the original suspension.

Muscle and Connective Tissue: About 200 Gm. of rat carcass devoid of internal organs, head and skin—containing, therefore, chiefly muscle, bones and connective tissue—was cut into pieces and dispersed in the Waring blender to a 50 per cent emulsion in saline solution. The emulsion was centrifuged at low speed. The supernatant was used as "muscle" and was presumably essentially free of connective tissue. The sediment, which consisted of large lumps and threads, was again run in fresh saline solution in the Waring blender and again collected by centrifugation. This process of washing was repeated six to ten times until a grayish white tough fibrous material was obtained. Then this was centrifuged at high speed to obtain it as dry as possible. The yield of this "carcass connective tissue" was roughly about 15 to 20 per cent of the original carcass material.

Grinding of this connective tissue was possible either in the frozen or in the dehydrated state. For grinding in the frozen state, pieces of the material about 5 to 7 mm. in diameter were placed in finely crushed carbon dioxide ice until solidly frozen. The material was then passed through an ordinary hand-driven flour mill which had previously been cooled by the passage of carbon dioxide ice alone. When the temperature of the ground material had risen above freezing, the connective tissue was suspended in saline solution in a mortar. In the more recent experiments, contact of the connective tissue with carbon dioxide ice was avoided because of the possibility that the antigens would be damaged by the concentrated carbon dioxide vapors. Instead of being frozen, the material was dehydrated either by being placed in small pieces in cold acetone, which was changed several times, and subsequently dried, or by being dried from the frozen state by the lyophil process. The dried material was passed once or repeatedly through the flour mill. It was finally suspended in saline solution in a mortar.

Subcutaneous Connective Tissue: The bulk of the fat was removed by placing small pieces of the material into cold acetone, which was changed several times. After drying, it was ground in the flour mill and suspended in saline solution in a mortar.

Preparation of the Streptococci.—Group A beta hemolytic streptococci (mostly of the strain Dochez N. Y. 5) were grown for forty-eight hours on a synthetic nonprotein medium according to the method of Bernheimer and associates,[5] collected by centrifugation, resuspended either in a small volume of the original culture fluid or in distilled water and immediately dried from the frozen state by the lyophil process. The yield was up to 1 Gm. of lyophil-dried organisms per liter of original culture. The organisms were kept in vacuo in the dark in tightly stoppered and paraffin-sealed bottles.

Preparation of Soluble Streptococcic Protein.—It seemed conceivable that when streptococcic protein in solution was admixed to the tissue emulsions rather than whole organisms, better conditions for the formation of antigen combinations between streptococcic and tissue substances might be obtained due to the higher degree of dispersion and the larger surface exposed in the case of soluble streptococcic protein.

For the preparation of streptococcic protein the organisms were ground according to the method of Mudd and others [6] in a low temperature ball mill, simplified for our purpose. As a grinding chamber, a heavy-walled pyrex glass centrifuge bottle

5. Bernheimer, A. W.; Gillman, W.; Hottle, G. A., and Pappenheimer, A. M., Jr.: J. Bact. **43**:495, 1942.

6. Mudd, S.; Shaw, C. H.; Czarnetsky, E. J., and Flosdorf, E. W.: J. Immunol. **32**:483, 1937.

of 250 cc. capacity was employed, which was stoppered by a tight-fitting rubber stopper containing, as an axle, a steel rod which was directly connected with the chuck of a speed-controllable electric motor. The grinding chamber was filled a little over one-half with $3/8$ inch (9 mm.) stainless steel balls. The apparatus was mounted with its axle in nearly horizontal position on a laboratory stand, and a tin can of suitable size was used as a "bearing" for the lower end of the grinding chamber, which was immersed in a "methyl cellosolven"–carbon dioxide ice cooling bath (about — 75 C.). Lyophil-dried living streptococci in the amount of 2 to 3 Gm. were ground at one time, the ball mill being run at 100 to 150 revolutions per minute for four hours. Then the cooling bath was removed, and after the contents of the chamber had reached a temperature above freezing, 50 cc. of distilled water was placed in the grinding chamber and the ground organisms were suspended by being rotated for a few minutes. The hydrogen ion concentration was brought to p_H 6.8 to 7 with tenth-normal sodium hydroxide. Then the ball mill was allowed to rotate for thirty minutes at room temperature. The suspension was collected with a pipet and there was a second washing with 50 cc. of distilled water. The combined suspension was then immediately cleared by high speed centrifugation in an angle centrifuge, and the supernatant, which contained a considerable amount of protein and which was tested for sterility, was immediately lyophil dried, frozen or admixed to the tissue emulsions and then dehydrated. The ground bacterial sediment, which microscopically was seen to consist of large masses of bacterial debris, also whole cocci, but no or few chains, was discarded.

Preparation of the Final Antigens to be Injected into Animals.—Freezing of the tissue or the tissue-streptococcus emulsions as a means of preserving them until use was highly unsatisfactory because the materials tended to aggregate in large clumps under these conditions. It was therefore found necessary to dehydrate the emulsions after grinding, which was carried out either by the lyophil process or by precipitation and drying with acetone. In a few instances the tissue-streptococcus emulsions were made up immediately before the beginning of the immunization schedule and the material was kept in the refrigerator.

The streptococci were added to the tissue emulsions and well mixed by a short run in the Waring blender. When the emulsion was to be lyophil dried, the streptococci were—previous to their being added to the tissues—killed by adding 10 per cent by volume of ether and letting them stand in the refrigerator overnight. On the other hand, when acetone drying was to be employed, living organisms were admixed to the tissue emulsions, as they were killed by the subsequent treatment. The drying with acetone was carried out as follows: The emulsion was added slowly, under stirring, to three to four times its volume of pure acetone which had been cooled to a temperature of about — 50 C. in a bath of carbon dioxide ice in methyl cellosolve or alcohol. After slow rise of the temperature to above freezing, the precipitate was collected by centrifugation, resuspended in acetone, again centrifuged, and so on. After three or four changes of acetone, the precipitate was easily dried under constant stirring at room temperature. While lyophil-dried tissues were kept in vacuo or in a desiccator, this did not seem necessary with acetone-dried materials.

The amounts of the dehydrated materials to be injected were weighed each day and suspended by grinding in a mortar, in distilled water in the case of lyophil-dried tissues or in isotonic solution of sodium chloride in the case of acetone-dried antigens.

Immunization Schedules and Doses.—With rats, as a rule, a schedule of ten intraperitoneal injections made on ten successive days was employed. With the

majority of the animals a second and often a third such schedule was used, intervals of one to two months being allowed between the schedules. In a few instances as many as six schedules were tried.

The standard dose of one schedule was the equivalent of 1 Gm. of fresh tissue (except in the case of connective tissue, in which 0.5 Gm. was employed) and 20 mg. of lyophil-dried streptococci. The soluble streptococcic protein was employed in amounts of 3 to 5 cc. per rat and schedule. Larger doses than this standard dose were not employed, but much smaller ones, about one tenth of the standard dose, were given in some cases. Further, in an attempt to determine the most effective proportion of streptococci to tissue emulsion, the amount of tissue emulsion was reduced to one third and one sixth, the dose of streptococci being kept constant. It seemed, however, from these experiments, that the standard dose and proportion were more effective.

Experimental Groups and the Antigens Employed for Their Immunization.— The following groups of rats were given the antigens listed.

Group 1 (135 rats). Rat heart plus streptococci (strain Dochez N. Y. 5):

(*a*) Rat heart plus streptococci (killed by ether), mixture lyophil dried (43 rats).

(*b*) Rat heart plus streptococci, acetone dried (58 rats).

(*c*) Rat heart, whole, plus soluble streptococcic protein (admixed fresh before each injection, mixture acetone dried) (12 rats).

(*d*) Extract of rat heart (the supernatant resulting from high speed centrifugation of rat heart emulsion) plus soluble streptococcic protein (kept frozen) (12 rats).

(*e*) Rat heart on which streptococci had been grown, acetone dried [7] (10 rats).

Group 2.—Rat carcass connective tissue plus streptococci (68 rats).

(*a*) Rat carcass connective tissue plus streptococci (killed by ether), lyophil dried (32 rats).

(*b*) Rat carcass connective tissue plus streptococci, acetone dried (15 rats).

(*c*) Rat carcass connective tissue, whole, plus soluble streptococcic protein (admixed fresh before injection; mixture acetone dried) (11 rats).

(*d*) Extract of rat carcass connective tissue (supernatant of centrifuged rat carcass connective tissue) plus soluble streptococcic protein (kept frozen) (10 rats).

Group 3. Rat subcutaneous connective tissue plus streptococci (35 rats):

(*a*) Rat subcutaneous connective tissue plus streptococci (killed by ether), lyoph'l dried (15 rats).

(*b*) Rat subcutaneous connective tissue plus streptococci, acetone dried (10 rats).

(*c*) Rat subcutaneous connective tissue, whole, plus soluble streptococcic protein (admixed fresh before injection) (6 rats).

(*d*) Extract of rat subcutaneous connective tissue (supernatant of centrifuged rat subcutaneous connective tissue) plus soluble streptococcic protein (kept frozen) (4 rats).

---

7. Each of 10 rat hearts was ground separately in 5 cc. of isotonic solution of sodium chloride, in a mortar, with the aid of sand. The emulsions were then heavily inoculated with a twelve-hour dextrose-broth culture of streptococci. After the mixture had been incubated for twenty-four hours at 37 C., all samples showed microscopically a fairly heavy growth of the streptococci. The samples were pooled, precipitated and dried with acetone.

Group 4.   Rat collagen plus streptococci (20 rats) :

(*a*)   Rat collagen from rat carcass connective tissue [8] plus streptococci, acetone dried (10 rats).

(*b*)   Rat collagen from rat tail tendon [9] plus streptococci, acetone dried (10 rats).

Group 5.   The fraction of rat carcass connective tissue which was soluble in 10 per cent sodium chloride solution, plus streptococci. This material represented the pooled extraction fluids from the first four extractions of rat carcass connective tissue with 10 per cent sodium chloride from the preparation of collagen from rat carcass connective tissue (see group 4 *a*) (20 rats).

(*a*)   The aforementioned fraction plus streptococci, acetone dried (10 rats).

(*b*)   The same fraction plus soluble streptococcic protein, acetone dried (10 rats).

Group 6.   Rat skeletal muscle plus streptococci (this material represented the portion of rat carcass which was readily emulsified in the Waring blender; presumably it consisted chiefly of muscle proteins and did not contain an appreciable proportion of connective tissue) (56 rats) :

(*a*)   Rat muscle plus streptococci (ether killed), lyophil dried (20 rats).

(*b*)   Rat muscle plus streptococci, acetone dried (16 rats).

(*c*)   Rat muscle, whole, plus soluble streptococcic protein, acetone dried (10 rats).

(*d*)   Extract of rat muscle (supernatant of centrifuged rat muscle emulsion) plus soluble streptococcic protein (kept frozen) (10 rats).

Controls.—The following groups of rats as controls were given the materials listed.

Group 7.   Rat heart (20 rats) :

(*a*)   Rat heart, lyophil dried (10 rats).

(*b*)   Rat heart, acetone dried (10 rats).

Group 8.   Rat carcass connective tissue (20 rats) :

(*a*)   Rat carcass connective tissue, lyophil dried (10 rats).

(*b*)   Rat carcass connective tissue, acetone dried (10 rats).

Group 9.   Streptococci (strain Dochez N. Y. 5) (52 rats) :

(*a*)   Streptococci, ether killed, kept frozen or lyophil dried (10 rats).

(*b*)   Streptococci, acetone dried (36 rats).

(*c*)   Soluble streptococcic protein (6 rats).

---

8. The collagen was prepared as follows: Rat carcass connective tissue was lyophil dried and then ground for about six hours in the low temperature ball mill · (in a manner similar to that described for the preparation of soluble streptococcic protein). After being ground, the material was extracted ten times for periods of twenty-four hours each with 10 per cent sodium chloride solution. After the sixth extraction, no protein was demonstrable in the extraction fluid with sulfosalicylic acid. Before admixture of the streptococci, the material was washed with distilled water and then suspended in isotonic solution of sodium chloride.

9. For the preparation of this material, the tendons of rat tails were collected and placed in 0.4 per cent acetic acid. A gelatinous solution of the collagen formed. The noncollagenous materials, which were not dissolved, were removed by centrifugation and gauze filtration.

Group 10. Rat kidney plus streptococci (this group represented animals which had been used for the experiments reported previously in which glomerulonephritis was produced).

Strains of Rats Used.—The majority of the rats employed were derived from the Evans strain, and all of them had been bred in our laboratory. Albino, gray, brown, black and hooded rats were used. The proportion of male rats immunized was slightly greater than that of female animals. Immunization was usually begun when the animals were between 2½ and 4 months old.

A smaller number of rats derived from another strain, namely, the Curtis-Dunning strain, were also used. These were chiefly immunized with rat heart plus streptococci. As will be pointed out later, the results with these animals were especially good. So far, not enough representatives of this strain have been available for use on a larger scale.

The room available for the rats had a relatively high temperature, averaging about 28 C. (82.4 F.). The diet consisted of a commercial dog chow [10] (about 60 per cent of the total) besides grains, bread, cabbage and carrots.

Technic of Serologic Tests.—For serologic studies, the rats were bled from the tail veins. The animals were first placed for a few minutes in a steam bath of about 45 to 55 C. The rat to be bled was then placed in a box from which its tail protruded. The vein of the distal half of the tail was punctured with a 25 gage needle. The flow of blood was kept up by an assistant who lightly massaged the tail in the distal direction. In this manner, 1.5 cc. of blood was relatively easily obtained. When more was taken, e. g., 2 cc., a few cubic centimeters of isotonic solution of sodium chloride was given intraperitoneally immediately after bleeding.

More recently another, more convenient method of bleeding was employed: The rat was etherized in a jar, but only to the point where complete anesthesia just started; i. e., as soon as the animal ceased to move, it was immediately removed from the ether jar. At this early stage of anesthesia the desired amount of blood could rapidly and easily be obtained from the ventral tail vein without any assistance or further manipulation. However, when anesthesia was allowed to proceed further, this satisfactory hyperemia of the tail disappeared, and bleeding was unsuccessful.

Bleeding was usually carried out on the seventh day after the last injection of the immunization schedule. In some rats repeated bleedings were made at intervals of five to seven days to determine the peak of antibody production.

---

10. The Ralston Purina Company states that the chow contains the following ingredients: meat meal, dried skim milk, riboflavin concentrate, carotene, cod liver oil, brewers' dried yeast, wheat germ, corn grits, wheat cereal, corn cereal, dried beet pulp, molasses, steamed bone meal, iodized salt.

Chemical analysis shows: protein (digestible) 19 per cent, fat (digestible) 4.7 per cent, fiber 4 per cent, ash 7 per cent, nitrogen-free extract (digestible) 48 per cent, moisture 7 per cent.

Mineral analysis shows:

Iron	0.018%	Sodium	0.67 %
Magnesium	0.09 %	Phosphorus	1.17 %
Silica	0.23 %	Chlorine	0.68 %
Potassium	0.56 %	Calcium	1.5 %

The vitamin content is described by the manufacturers as follows: Vitamin A, about 4,000 U. S. P. units per pound; vitamin B, about 275 Sherman units per pound, supplied by wheat germ; vitamin C, low; vitamin D, about 500 U. S. P. units per pound; vitamin E, supplied by wheat germ; vitamin G, 300 Sherman units per pound.

Because of its high sensitivity, the collodion particle technic was employed for practically all the serologic work.

The antigens used for these tests were extracts obtained in isotonic solution of sodium chloride (0.85 per cent NaCl) from the frozen or freshly ground 20 per cent tissue emulsions, prepared by centrifugation of the emulsions. The supernatants were usually frozen. Freezing did not seem to weaken the activity of the extracts, but it greatly helped to clear them. The extracts for serologic testing were always made from a batch of emulsion made up from a pool of the same tissues from different animals.

Summary of Serologic Results *

Treatment	Rats from Which Serologic Results Were Available	Rats with Low or Moderate Titers (Positive Agglutination in Serum Dilutions up to 1:40 or 1:80)	Rats with High Titers (Positive Agglutination in Serum Dilutions of 1:160 or Higher; up to 1:1,280)	Sum Total of Rats with Auto-antibodies (Positive Agglutination in Serum Dilutions of 1:40 or Higher)	Negative
Evans strain rats: rat heart plus streptococci	74	21	19	40	34
Among these: rat heart plus streptococci, acetone dried	22	7	3	10	12
Curtis-Dunning strain rats: rat heart plus streptococci, acetone dried	30	12	14	26	4
Rat subcutaneous connective tissue plus streptococci........	25	5	7	12	13
Rat carcass connective tissue plus streptococci	26	6	16	22	4
Rat skeletal muscle plus streptococci	22	8	6	14	8
Streptococci (killed) (tested with rat heart as antigen).....	32	1	0	1	31
Rat heart	10	0	0	0	10
Rat carcass connective tissue..	10	0	0	0	10

* Recorded is the highest titer observed with the homologous tissue antigen in each rat. With most of the rats serologic tests were carried out repeatedly, i. e., after each immunization schedule.

The tests were set up as follows: Serum dilutions were made from each serum in amounts of 0.5 cc., the doubling dilution being used, usually beginning with a dilution of 1:10. Collodion-antigen mixture (0.2 cc.) was added to each tube. The contents were mixed by shaking, and the tubes were incubated at room temperature for one to two hours. The tubes were then centrifuged in an International Equipment Company centrifuge (horizontally) at 1,400 revolutions per minute for exactly three minutes. The results were read under an artificial light and in front of a black screen while the tubes were carefully shaken, and the sizes of the agglutinations were noted. They were recorded from plus-minus ($\pm$) to 4 plus ($++++$). The collodion particles were prepared as described elsewhere.[11]

---

11. Cavelti, P. A.: J. Immunol. (*a*) **49**:365, 1944; (*b*) to be published.

For sensitization, the particles were admixed to the antigen immediately before use. In order that nonspecific reactions might be reduced to a minimum, the antigen was diluted to the final dilution (usually 1:15 or 1:20) before addition of the particles. Then enough of the stock collodion was added to make a final dilution of 1:20, and the mixture was ready for being admixed to the serum dilutions.

In most of the tests, about 6 to 10 normal rat serums were run as controls besides serums from rats treated with control antigens, e. g., streptococci alone or tissue emulsions alone. For each batch of collodion particle suspension it was ascertained that the particles alone were not agglutinated by normal or immune rat serum. Antigen controls were set up with saline solution instead of serum.

RESULTS OF THE SEROLOGIC STUDIES

Serologic studies were carried out with a considerable proportion of the experimental animals' to determine whether antibodies were formed which reacted with the tissue component of the tissue-streptococcus antigens used for immunization. It should be emphasized that for the in vitro tests, extracts of plain normal tissues were used throughout, i. e., not in combination with streptococci or their products.

Part of the studies were carried out on pools of serums from 5 rats similarly treated. However, the results with pooled serums were markedly inferior to those obtained when individual serums were tested.

Whenever possible, the serologic tests were done not later than twenty-four hours after collection of the serums, as the autoantibodies were found not to be stable when the serums were stored. When serums were retested after a week or two, the titers were considerably lower. However, autoantibodies were still demonstrated in some of the stronger serums after a storage of several months.

Together with the testing of serums from experimental animals, control serums were also tested, which usually were taken from the animals on the same day. These controls comprised samples from normal untreated rats and from rats treated with streptococci alone and tissue emulsion alone. For many of the tests serums were also obtained from the experimental animals before immunization was started, for use as controls.

Some difficulties were encountered regarding nonspecific flocculation of the sensitized collodion particles. This was revealed by flocculation of the antigen in the presence of the control serums. Many of these false reactions could, however, be eliminated in the later tests by an improvement of the collodion technic, described in detail elsewhere.[11b] Some of the tissue antigens proved to be entirely unusable. Collagen, both that from rat carcass connective tissue and that from rat tail tendon, is insoluble in isotonic solution of sodium chloride and thus cannot be tested by the collodion technic. The extract of rat carcass connective tissue in 10 per cent sodium chloride solution was tested in a setup in

which 10 per cent sodium chloride solution was used throughout, also for the serum dilutions; however, the reactions were weak and not considered conclusive. With other antigens and serums known to react positively it was found that 10 per cent sodium chloride solution as a medium greatly inhibited the reactions.

The extracts of carcass-connective tissue and of subcutaneous connective tissue in isotonic solution of sodium chloride appeared to yield conclusive results in some large scale tests, as all the control serums were negative, while at other times much nonspecific flocculation was encountered for reasons not yet understood.

Considerably fewer nonspecific reactions were seen with extract of rat skeletal muscle, although here, too, some difficulties were experienced. The most reliable results were obtained with rat heart extract as antigen. However, in the earlier part of the studies, false positive reactions occurred occasionally with serum dilutions up to 1:20. Therefore, in all tests, only reactions with serum dilutions exceeding 1:20 were considered positive.

Generally, as seen in the accompanying table, the results of the serologic studies show that in a considerable proportion of the animals treated with rat tissue emulsions in conjunction with killed streptococci, antibodies were demonstrable which reacted in vitro with extracts of the plain homologous tissue component. The serums from rats treated with either tissue emulsion alone or with killed streptococci alone failed to show any evidence of such antibodies. The titers of the autoantibodies of the tissue-streptococcus treated groups ranged from 1:40 to 1:1,280 (serum dilutions in which positive agglutination appeared). The highest titers were usually found with the samples taken on the seventh day after the last injection. With samples taken subsequently at five to seven day intervals the titers were considerably lower; however, at times autoantibodies were still demonstrable several weeks after immunization. The titers and the incidence of autoantibodies were found to be much higher for the same rats after the second or third schedule of immunization than after the first.

The mode of preparation of the antigenic mixtures appeared to be of considerable importance with regard to the strength of the resulting in vitro reactions. Thus mixtures of tissue emulsion and whole streptococcic cells were the most effective preparations. Although serologically there did not seem to be a definite difference in effectiveness among acetone-precipitated, lyophil-dried or raw mixtures, the use of acetone-precipitated materials seemed preferable in certain other respects, as discussed later. The antigenic effect of mixtures of tissue emulsion and soluble streptococcic protein seemed considerably weaker than that observed when whole streptococcic cells were used. The animals treated

with extracts of tissues (instead of emulsion) in conjunction with soluble streptococcic protein failed to show any evidence of autoantibody production.

The strain of rats appears to be of interest, too, as the representatives of the Curtis-Dunning strain treated with heart-streptococcus antigen had titers far outreaching those of the Evans strain, both in height and in incidence. The agglutinations in the individual tubes were also unusually intense.

In attempts to investigate the specificity of the autoantibodies produced to the various tissues, a considerable number of the serums were tested with heterologous tissue extracts, i. e., with extracts of rat tissues other than the ones used for immunization in each particular case. Thus a number of serums from rats treated with streptococci plus heart, streptococci plus connective tissue, and streptococci plus skeletal muscle were tested with each of these three tissue antigens. As might have been expected, the cross reactions observed were extensive. There was, however, no reaction with any of these serums when they were tested with rat kidney. The serums also failed to react with extracts of rat liver or spleen, while they showed slight reaction with extracts of rat lung.

PRODUCTION OF AUTOANTIBODIES TO HEART IN RABBITS

In the early stage of the studies some rabbits also were immunized with mixtures of killed streptococci and emulsion of perfused rabbit heart. The components were admixed in the proportion of 2 parts of 20 per cent emulsion of heart to 1 part of a suspension of ether-killed streptococci containing 2 per cent by volume of packed streptococcic cells (corresponding to about 4 to 4.5 mg. of lyophil-dried organisms per cubic centimeter). The beginning doses were 1 to 2 cc. of the mixture. The injections were given intraperitoneally either daily or at intervals of three to five days, and the doses were gradually increased to 6 to 7 cc. After every five injections a rest period of several weeks was allowed. The maximum number of injections given was fifteen, administered in three schedules of five injections each. A total of 14 rabbits was so treated. Evidence of production of antibodies reacting in vitro with extracts of plain rabbit heart was obtained. The serums of 7 of these rabbits showed fairly strong reactions in serum dilutions of 1:80 or higher, the serums of 4 rabbits gave weak or doubtful reactions, and those of the remaining 3 were always nonreactive when tested. Some of the strongly reacting serums also reacted strongly with extracts of rabbit skeletal muscle, weakly with rabbit lung and not at all with rabbit kidney, liver, spleen or brain.

COMMENT

The results of these experiments indicate that when rats are given injections of mixtures of killed streptococci and emulsions of certain rat tissues, such as heart, skeletal muscle and connective tissue, antibodies are formed which react in vitro with extracts of the tissue component used for immunization. As such antibodies cannot be produced

by injections of the homologous tissue emulsions alone (i.e., without streptococci), the streptococcus obviously has the capacity to render certain substances of these tissues antigenic for the homologous species, presumably by some loose combination between streptococcic and tissue substances. The antibodies formed have the ability to react with the tissue component alone (i.e., without the presence of streptococcic substances). The tissue components may therefore be spoken of as haptens. From the results reported, these antibodies would logically be termed isoantibodies. However, as will be shown in "Studies on the Pathogenesis of Rheumatic Fever: II," they, or at least some of them, appear to be able to cause tissue damage by means of their reaction in vivo with the tissue of the same animal. The term "autoantibody" is therefore applicable. Formation of such autoantibodies to heart has also been demonstrated in some rabbits which were immunized with killed streptococci plus rabbit heart.

Presumably a multiplicity of autoantibodies are formed by the procedures used in these experiments as well as those reported previously concerning the formation of autoantibodies to kidney. The autoantibodies to heart, skeletal muscle and connective tissue show a great deal of overlapping in vitro. This is not surprising, as connective tissue, for instance, is present in both heart and skeletal muscle; in fact, most of the connective tissue used in these experiments was prepared from skeletal muscle. While the skeletal muscle antigens used were thought to be largely free of connective tissue, the preparations of connective tissue, in turn, could hardly be free of skeletal muscle antigens.

Some of the tissue antigens employed for injection in conjunction with streptococci, such as preparations of collagen and extract in 10 per cent sodium chloride solution of connective tissue, could not be tested serologically by means of the collodion method, as they are insoluble in isotonic solution of sodium chloride. One would probably have to resort to complement-fixation technics for this purpose.

SUMMARY

Rats were given injections of mixtures of killed group A beta hemolytic streptococci and rat heart, rat skeletal muscle and various preparations of rat connective tissue. Autoantibodies to these tissues were formed, which could be demonstrated in vitro with the collodion particle technic and use of extracts of plain homologous tissues.

STUDIES ON THE PATHOGENESIS OF RHEUMATIC FEVER

II. Cardiac Lesions Produced in Rats by Means of Autoantibodies to Heart and Connective Tissue

PHILIP A. CAVELTI, M.D.

SAN FRANCISCO

IN THE preceding paper [1] ("Studies on the Pathogenesis of Rheumatic Fever: I") evidence has been presented that autoantibodies to heart, connective tissue and skeletal muscle can be produced in rats and rabbits by immunizing the animals with emulsions of the homologous tissues in conjunction with killed streptococci. Streptococcic materials obviously have the ability to render autogenous tissue substances antigenic, thus leading to formation of autoantibodies which react specifically with the respective tissue components. This fact having been ascertained, the question arose whether or not these autoantibodies, by combining with the antigen situated in the tissues in vivo, are capable of acting as a pathogenic agent, leading to damage of these tissues. As already mentioned, these experiments were undertaken with a view to the genesis of rheumatic fever. The organ chiefly examined with respect to such lesions was, therefore, the heart.

METHODS

Most of the materials and methods used in these experiments, such as the tissue and streptococcus antigens and the methods of immunization, have been described in the preceding paper [1] and that description need not be repeated here.

Pathologic Procedure: The rats were killed with ether in groups at various intervals, from seven to one hundred and twenty days, after the immunization schedule, or after various numbers of such schedules, had been carried out. A small portion of the animals died spontaneously after various intervals. The tissues were placed in 4 per cent solution of formaldehyde immediately after the animals had been killed (or as soon as possible after spontaneous death). In most instances the weight of the body, that of the heart and that of both kidneys were recorded. In the earlier part of the experiments the heart was opened and the valves were inspected. However, as the gross changes of the valves were difficult

1. Cavelti, P. A.: Arch. Path., this issue, p. 1.

to evaluate, and especially because much better histologic sections were obtained when the heart was not opened before fixation, this was discontinued. Subsequently, the heart, after being removed from the body, was squeezed between absorbent cotton pads to remove most of the blood from the cardiac chambers and then was placed in solution of formaldehyde U.S.P. After a few days, the heart was cut in half longitudinally in such a way that the mitral and aortic valves were sectioned at the same time. Histologic sections were then made from both halves of the organ.

RESULTS

Gross Pathologic Findings.—With regard to changes referable to the effect of autoantibodies, little of note was seen on gross inspection. In a few instances, slight epicardial deposits of fibrin were visible, chiefly over the auricles and occasionally localized in narrow bands over the upper portions of the ventricles and over the region of the atrioventricular border. Sometimes subepicardial hemorrhages were noted in the region of the ventricles. Some of the hearts appeared to be dilated, especially the auricles, but no reliable standards of reference have been established by which to ascertain this. The cardiac weight, although frequently increased, failed to reveal much with respect to the histologic changes subsequently found. In some of the cases in which the heart was opened, more or less definite verrucous changes were seen in the valves, especially the mitral valve. However, as already mentioned, accurate observations were difficult because of the smallness of the object, and gross inspection of the valves was discontinued prior to fixation in favor of better histologic sections.

Occasional gross changes of other organs were: petechiae and larger hemorrhages of the lungs. This was noted in a number of animals killed relatively early after the immunization schedule had been carried out, i. e., ten to twenty days after the last injection. In other rats the lungs showed spotty pneumonic consolidation and, infrequently as a spontaneous lesion, pulmonary disease with abscess formation.

Enlargement of the spleen was a frequent finding, especially in rats killed relatively soon after treatment.

As to lesions due to the local effect of the injected material, peritoneal inflammatory reaction is to be mentioned. The early stage of this reaction was in the form of fibrin deposit on the peritoneum, with or without a small amount of fluid. Later, adhesions were observed, and in cases in which the injury was severe, abscess formation. In some rats, multiple abscesses, occasionally even with perforation of the abdominal wall, were noted. Cultures of material from these abscesses either proved sterile or revealed contaminating organisms, never streptococci. Peritonitic changes of the sort described were relatively common in rats that had received repeated large doses of raw or lyophilized streptococcus-tissue antigens, especially those which contained a large amount of coarse material and in which the streptococci had been killed by ether.

Such changes, with the exception of slight adhesions, were, however, rare in rats which had been given streptococcus-tissue antigens that had been precipitated and dried with acetone, as well as in those which had been given predominantly soluble materials or fine emulsions. The connective tissue antigens were not obtained sterile and during their preparation further contamination was almost unavoidable. Treatment of these materials with acetone practically sterilized them, which probably accounts to a large extent for the low incidence of peritonitic changes resulting from their use. A further possibility is that the treatment with acetone had a detoxifying effect on the streptococcus and tissue components.

When the incidence of peritonitic changes was compared with that of cardiac lesions in each individual rat, there was no parallelism between the two. There seemed to be rather a slight tendency toward the opposite.

A few rats had abscesses in many organs, including the heart. In some cases these changes seemed to have originated from spontaneous pulmonary lesions; in other instances, from contaminated materials injected intraperitoneally. The lesions of these animals were excluded from the evaluation of the results of the present studies.

Histologic Changes Observed in Heart.—Generally speaking, the lesions to be described involved chiefly the connective tissue structures of the heart, i. e., valves, valve rings and adjacent regions, the parietal subendocardial connective tissue layers, the interstitial and perivascular connective tissue of the myocardium of ventricles and auricles, and the epicardium.

(*a*) Valves and valve rings: Most commonly involved was the mitral valve, somewhat less commonly the aortic valve, often both, and rarely the tricuspid or the pulmonary valve.

The lesions were evidenced chiefly by infiltrative and proliferative changes. Sometimes they were distributed more or less diffusely over most of the valves, but more often their incidence was accentuated in certain areas, such as the tip and other exposed portions of the valve. They also were commonly found in the region of the valve ring. While some of these lesions gave the impression of mere cellular accumulations, others took the form of more or less definite nodular structures.

There was infiltration in which lymphocytes, some leukocytes, monocytes and plasma cells participated. However, a considerable part of the increased cellularity was usually due to fibroblastic proliferation and to the appearance of relatively large cells with a more or less definite tendency toward basophilia. Sometimes the basophilic protoplasm of the latter showed ragged and somewhat indefinite outlines. The nuclei were usually more or less compact and slightly elongated. Pyknotic and fibrocytoid nuclei were, however, sometimes present, and owl-eyed nuclei

Fig. 1.—*A*, normal mitral valve leaflet of a rat treated with rat skeletal muscle plus streptococci; × 100.

B, inflammatory reaction in the mitral valve of a rat treated with rat carcass connective tissue and streptococci; × 100.

C, inflammatory and proliferative reaction, with considerable thickening, of the mitral valve of a rat treated with rat subcutaneous connective tissue and streptococci; × 100.

D, area of proliferative cellular reaction near the base of the mitral valve of a rat treated with rat heart and streptococci; × 450.

were also noted, though infrequently. Some of the cells of the type described were multinuclear. In a portion of the cases there were apparently degenerative changes of the connective tissue, consisting of swelling and fragmentation of the ground substance (collagen?) with increased eosinophilia. These findings were sometimes associated with more or
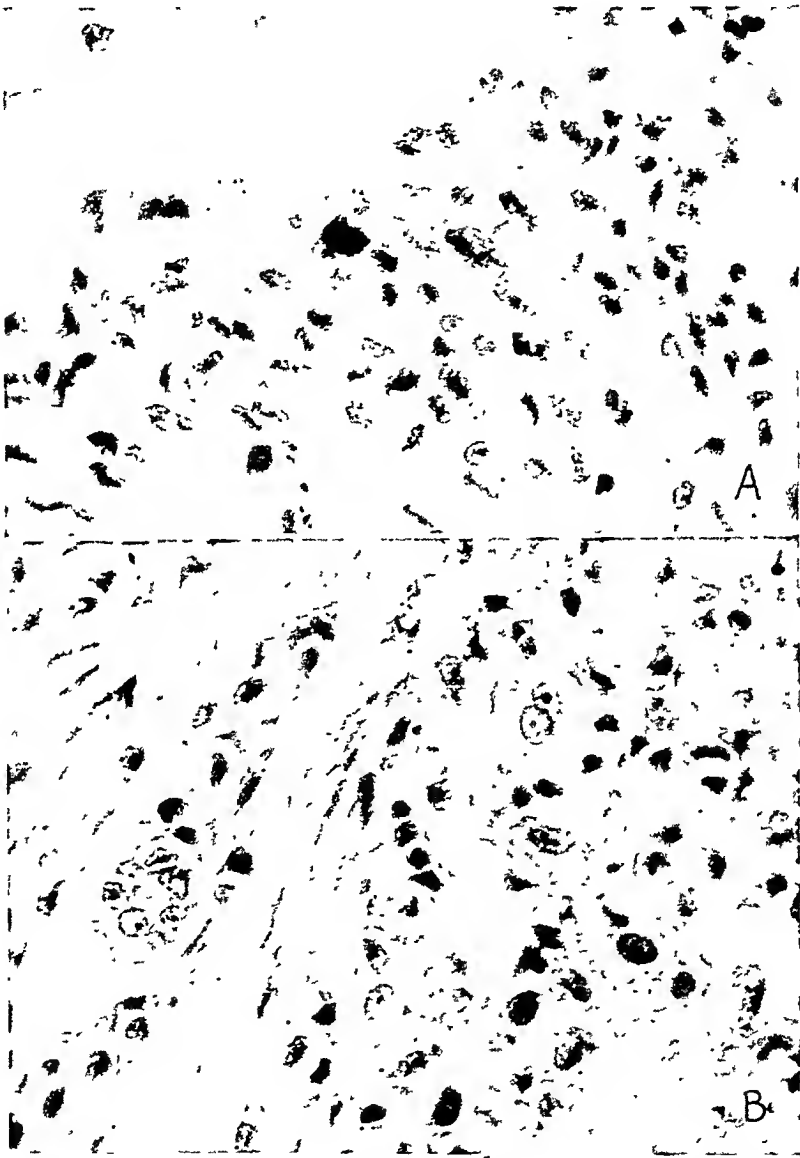
Fig. 2.—*A*, cellular reaction in the valve ring of a rat treated with rat carcass connective tissue and streptococci; × 500.

B, cellular reaction in the valve ring of a rat treated with rat heart plus streptococci; × 500.

less definite cellular nodule formation. As to lesions of the type described, many variations were seen. While granulomatous structures consisting chiefly of somewhat large and occasional multinuclear cells

with a tendency toward basophilia were seen relatively often, it is doubtful whether any of these structures can be considered to resemble closely typical Aschoff bodies. In most of the cases, either the nuclear formation typical of Aschoff cells or conspicuous collagen degeneration was lacking. These lesions were sometimes protruding near the tip of the valves or in other exposed regions besides being located deeper in the valve or in or near the valve ring. Transitions to structures leading to scarring, i. e., with an increased proportion of fibroblasts and fibrocytes, up to lesions made up chiefly of such cells were seen. Some of the valves, therefore, showed more or less definite thickening, either confined to certain portions or involving the whole of a leaflet. In a few cases such thickening was conspicuous. Deformation of the thickened leaflets, although in some cases undoubtedly present, was usually difficult to evaluate from histologic sections only. Vascularization occurred in some of the valves showing considerable proliferative changes. Exudative changes were uncommon. In some cases marked edema of the valves, associated with infiltration, was present. Thrombi were rarely seen on the valves.

(*b*) Parietal endocardium: The changes consisted of edema and thickening of the subendocardial connective tissue layer with varying degrees of inflammatory cell infiltration of this layer. The endocardium itself sometimes showed endothelial proliferation. These changes were not common and were mostly confined to the parietal endocardium of the auricles, especially to that of the left auricle.

(*c*) Myocardium: As already mentioned, the myocardial changes also were predominantly confined to the connective tissue, i. e., the interstitial and perivascular connective tissue. All variations from mere lymphocytic infiltrations to focal and more or less definite nodular lesions of the type described for the valves were noted. In some cases the interstitial and perivascular cellular lesions were impressive and widespread and in later stages associated with a considerable degree of interstitial and perivascular fibrosis resulting in destruction of the adjacent muscle fibers. In some of these instances widespread myocardial scarring, partly with cellular lesions within the scars, was notable. However, on the whole, these myocardial changes were less commonly encountered and less intense than the valvular lesions. When present, the interstitial and perivascular changes were usually more pronounced in the upper portion of the ventricle than near the apex. In some cases they were also outstanding in the auricles.

Involvement of the walls of small arteries and arterioles was not frequently observed. Occasionally there seemed to be some degree of medial infiltration and slight proliferation and hyalinization of the endothelium of small vessels. In some animals more pronounced changes of this sort were noted in the walls of large arteries or in that of the aorta
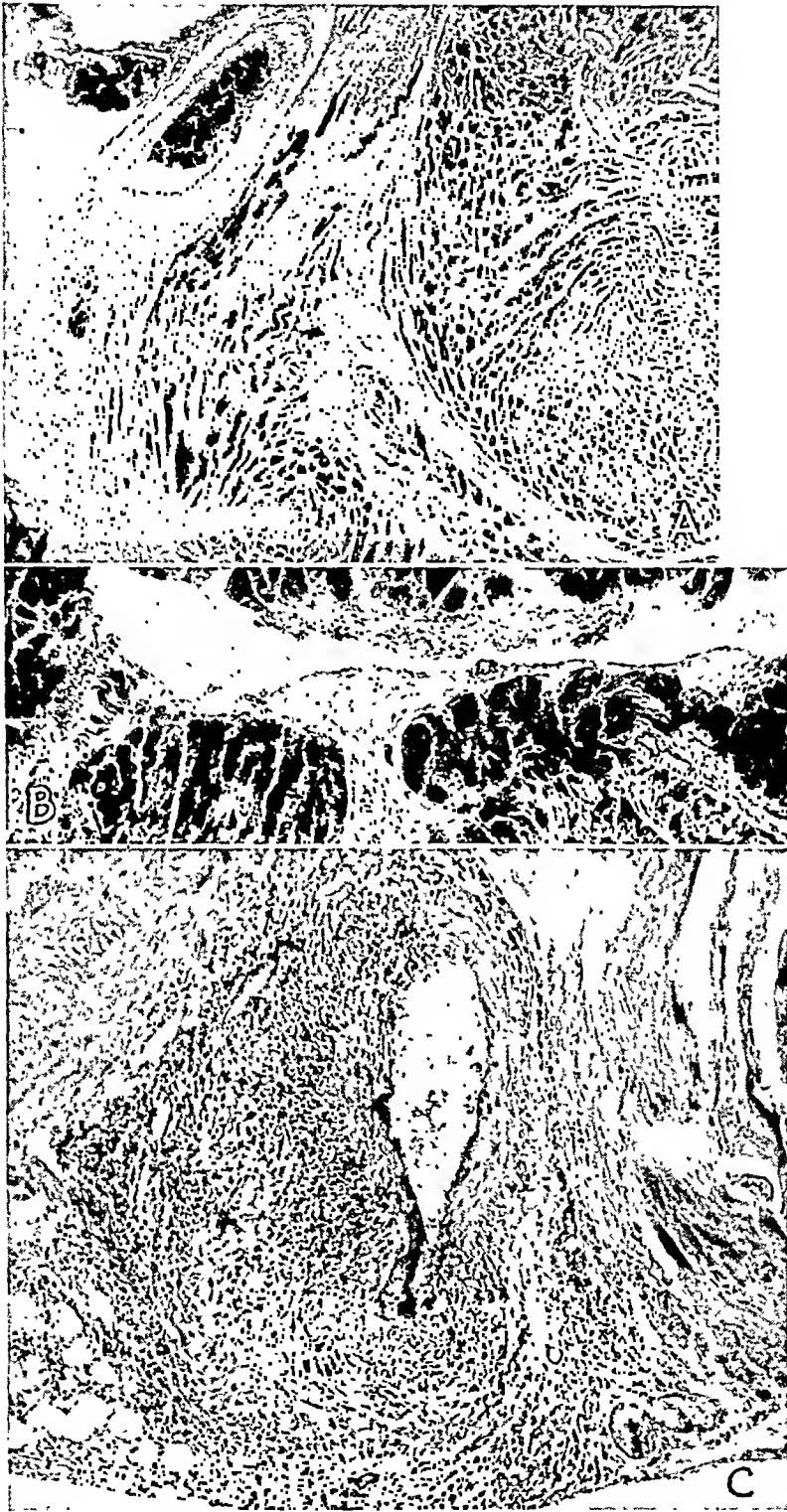
Fig. 3.—*A*, interstitial and perivascular inflammatory reaction and scarring in the upper left ventricular region of a rat treated with rat heart and streptococci; × 50.

B, ·perivascular scarring in a rat · treated with rat heart and streptococci; × 100.

C, arteritis and periarteritis of a medium-sized vessel of a rat treated with rat carcass connective tissue plus soluble streptococci protein; × 100.

near its root, varying in degree up to a striking infiltration of the whole arterial wall with inflammatory cells associated with endarteritis and periarteritic nodule formation.

(*d*) Epicardium: Typical exudative or adhesive pericarditis was not, observed, and the pericardial sac was not included in the histologic sections. However, in a few cases the epicardium showed a more or less prominent layer of fibrin with an infiltrate of inflammatory cells. Occasionally small nodular structures were also present. There were varying degrees of organization of the epicarditic layer. These changes were mostly confined to the auricles and were present only in some parts of the auricles; however, in a few instances a more or less continuous epicarditic layer extending around the auricles and a considerable part of the ventricles was noted.

Changes in Other Organs.—Owing to technical circumstances, it was not possible to study other organs histologically on any large scale. However, sections were obtained from lung, liver, spleen, kidney and skeletal muscle (the latter from the region of the elbow) from a small number of rats from each of the major experimental groups.

The most important changes found were perivascular inflammatory reactions. These were common, especially in liver, lung and kidneys, and consisted of collections of inflammatory cells in close proximity to the vessels, particularly arterioles. Some of these collections showed more or less the appearance of small nodules; however, degenerative changes of collagen were not conspicuous, nor were the cells generally of a type comparable to Aschoff cells. Quite common were broad sheaths of inflammatory cells (lymphocytes, plasma cells, monocytes, also some eosinophilic leukocytes) embracing the whole vessel, accompanied in part of the cases by medial infiltration and slight intimal proliferation. Some of these lesions resembled periarteritis nodosa. Sometimes similar sheaths of inflammatory cells were present also around bronchioles in the lungs. In some cases further thickening and slight infiltration of the alveolar wall were seen, and some of the alveoli were occasionally filled with leukocytes and lymphocytes as well as erythrocytes. Slight perivascular reaction was also noted in some of the sections of skeletal muscle but it was not severe or widespread. Interstitial cellular accumulations or nodules were encountered in skeletal muscle in some cases. In a few instances liver and skeletal muscle showed slight perivascular scarring. Sections from spleen generally failed to reveal any significant changes.

Clinical Observations.—Clinical observations revealed little more than the fact that some of the rats, especially during a period of about one to two weeks after the immunization schedules had been carried out, appeared generally sick. Some of these rats died during this period.

No way of estimating clinically the presence and the intensity of cardiac lesions of rats has as yet been developed. Auscultation of the heart failed to yield conclusive findings. Electrocardiograms have recently been taken on some rats, and if the changes prove significant, they are to be reported later.

Arthritis was generally not observed. Routine histologic studies of the joints were, however, not performed. One rat of the Curtis-Dunning strain on which one injection schedule had been carried out with heart-streptococcus antigen precipitated and dried with acetone showed, ten days after the last injection, marked swellings of several joints, especially of the elbows, the knees and the hind feet. The animal appeared very sick, and the swollen joints were obviously painful. The swellings disappeared within about four days. Because of a mishap, the joints were not available for pathologic study. However, the heart of this animal, which was killed because of emaciation two months after the treatment, was one of those showing the most intense changes, especially numerous small interstitial and perivascular scars with and without cellular reaction, further nodular cellular reactions, and thickening of the mitral valve.

EVALUATION OF THE CHANGES OBSERVED IN THE VARIOUS EXPERIMENTAL GROUPS

The types of cardiac lesions were tabulated for each rat, and the intensity of the changes was recorded from plus-minus ($\pm$) to 4 plus ($+ + + +$). Changes which were unquestionably nonspecific and which were also found in normal untreated rats were excluded from this evaluation. The latter changes consisted chiefly of slight lymphocytic infiltrations. It semed desirable to determine, on the one hand, the incidence of all possibly significant lesions, including the slight ones (i. e., those recorded at 1 plus or better) and, on the other hand, the incidence of only the well developed lesions (2 plus or more). The accompanying table shows the percentage incidence of these lesions in the various experimental and control groups. It can be seen that the incidence of cardiac lesions is significant in the groups treated with heart-streptococcus and connective tissue–streptococcus antigens while being negligible in the control groups—whether the evaluation is made on the basis of all, including slight, changes or on the basis of only the more marked ones. The fact that no cardiac lesions resulted from treatment with either streptococci alone or heart or connective tissue alone supports the view that the lesions obtained when these tissues were injected in conjunction with streptococci were due to the autoantibodies found under these conditions. As described in the preceding paper,[1] autoantibodies were obtained essentially only when combinations of streptococci and tissue emulsions were injected, but not on treatment with either of the components alone. The function of the streptococci

obviously is to render autogenous tissue material antigenic. A further strong argument in favor of the pathogenic action of the autoantibodies produced would be to show that there is an interval between immunization and onset of the lesions corresponding to the time necessary for the formation of the autoantibodies. However, as repeated schedules of immunization were carried out on the great majority of the animals, this point could not be readily ascertained. From the little evidence there is, it seems that the changes did not appear before about the seventh to tenth day after the last injection of the primary schedule. A related question would be whether repeated treatment increases the incidence of cardiac lesions. The few rats from which histologic preparations are available after one injection schedule are animals which had died or were killed when very sick apparently as a result of the treatment or its sequences. The incidence of lesions can therefore be expected to be relatively too high in this group. Actually it was about the same as in the group of rats killed after several treatments. The fact, however, emerges from the results that more than three schedules of immunization failed to increase further the incidence of cardiac lesions, although the intensity of the lesions may have increased.

As to the correlation between the titer of autoantibodies and the extent of the cardiac lesions, the results show that there is a fairly good correlation between the incidence and the titer of autoantibodies to heart and to connective tissue when the various experimental groups are compared as a whole. Thus, for instance, rats from the Curtis-Dunning strain responded to heart-streptococcus antigen with much higher titers of autoantibodies and also showed more definite cardiac lesions than the regular strain of rats. Furthermore, the animals treated with soluble streptococcic protein mixed with either heart or connective tissue showed a low degree of serologic reaction and an equally low tendency toward cardiac lesions. However, when the serologic results from individual rats were compared with the histologic ones, the correlation was not so evident in all cases. But this could hardly be expected, as the serologic tests were carried out too infrequently, and in many rats there was an interval of many months between the last serologic test and autopsy.

A noteworthy fact is that cardiac lesions failed to develop in the animals treated with streptococci plus skeletal muscle essentially devoid of connective tissue, although these animals had autoantibodies reacting in vitro with extract of skeletal muscle. This type of autoantibody, therefore, seems to lack pathogenic effects, or it may, owing to the large amount of muscle tissue present in the body, be so highly dispersed that it is insufficient to produce lesions in any one locality.

The evidence available concerning the mechanism of origin of the cardiac lesions produced supports the view that these lesions are due

to the pathogenic effect of the autoantibodies produced when the animals are immunized with autogenous tissues, especially heart and connective tissues, in conjunction with streptococci. An important question arises: In which tissue is the antigen located which incites those autoantibodies that are responsible for the cardiac lesions? From the table it is evident that cardiac lesions were obtained when either heart or connective tissue was used in conjunction with streptococci. In contrast to this, no lesions resulted from the treatment with streptococci plus skeletal muscle largely devoid of connective tissue. The heart antigens used in our experiments undoubtedly contained a large proportion of connective tissue, as care was taken not to lose any of this fraction during the preparation of the antigens. As yet, no animals have been treated with cardiac connective tissue devoid of most of the muscular portion of heart in conjunction with streptococci. However, the results suggest that with the use of heart, too, the connective tissue contained therein may be the site of the antigen in question.

From the results with the two separated fractions of connective tissue (in conjunction with streptococci), i.e., the portion of carcass connective tissue which is soluble in .10 per cent sodium chloride solution, on one hand, and the portion which is insoluble (representing chiefly collagen), together with collagen from rat tail tendon, on the other, nothing definite can be concluded, as the groups are too small and the histologic changes obtained too slight. The fact that the incidence of interstitial myocardial changes was slightly higher in the groups treated with rat kidney plus streptococci than in the other control groups may be explained by the presence of some connective tissue or a related antigen of the kidney. More likely, however, this higher incidence is the result of the inclusion in this group of some very old rats with long-standing chronic glomerulonephritis; in such rats the myocardial lesions may be of a different nature. Also, some of these rats had received exceedingly large amounts and many schedules of injections of streptococcus-kidney antigen, partly also living streptococci. It is conceivable that under such conditions an in vivo formation of streptococcus-tissue antigen might be effected with subsequent production of autoantibodies of the type under study, and consequent cardiac lesions. That in principle the possibility of such mechanisms exists will be shown in another paper.[2]

There did not seem to be any significant difference in the nature of the histologic lesions obtained by treatment with mixtures of either

---

2. Cavelti, P. A.: Studies on the Pathogenesis of Glomerulonephritis and Rheumatic Fever: In Vivo Activation of Tissue Antigens as a Result of Streptococcic Infection, and Consecutive Formation of Autoantibodies, Arch Path., to be published.

Incidence of Cardiac Lesions

Material Injected	Rats	Cellular Reaction of Valves 1+	2+	Thickening Scarring of Valves 1+	2+	Cellular Reaction of Interstitial and Perivascular Connective Tissue of Myocardium 1+	2+	Scarring of Interstitial and Perivascular Connective Tissue of Myocardium 1+	2+	Granulomatous Lesions 1+	2+	Pericarditis 1+	2+	Arteritis, Periarteritis, Endarteritis 1+	2+
							Percentage Incidence of Histologic Changes								
1. Rat heart + streptococci, Curtis-Dunning strain rats	37	51	32	25	16	54	27	8	5	35	11	3	0	3	0
2. Rat heart + streptococci, Evans strain rats	54	45	22	11	7	26	7	13	4	7	0	15	7	17	13
3. Rat heart + soluble streptococcic protein	21	10	0	10	0	10	10	0	0	0	0	0	0	10	5
4. Rat subcutaneous connective tissue + streptococci	31	29	16	20	10	20	16	13	7	10	10	13	0	7	3
5. Rat carcass connective tissue + streptococci	44	46	40	22	18	30	18	20	11	18	13	13	9	0	9
6. Rat carcass connective tissue + soluble streptococcic protein	20	25	10	5	0	0	0	10	0	10	5	0	0	15	10
7. Rat carcass connective tissue, 10% NaCl soluble fraction + streptococci	10	30	20	20	20	20	10	20	20	20	10	0	0	0	0
8. Rat carcass connective tissue, 10% NaCl soluble fraction + soluble streptococcic protein	10	20	10	0	0	10	10	0	0	10	0	0	0	0	0
9. Rat carcass connective tissue collagen + streptococci	10	10	0	20	10	10	0	10	0	0	0	0	0	10	10
10. Rat tail tendon collagen + streptococci	10	10	10	0	0	10	0	10	0	0	0	0	0	0	0
11. Rat skeletal muscle + streptococci	45	4	2	2	2	0	0	0	0	0	0	0	0	2	2
Controls:															
12. Rat kidney + streptococci	57	5	2	3	3	10	2	12	2	3	2	0	0	2	0
13. Streptococci, killed	16	0	0	0	0	0	0	0	0	0	0	0	0	6	6
14. Streptococci, living	10	10	0	10	10	10	0	0	0	0	0	0	0	0	0
15. Rat heart	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16. Rat carcass connective tissue	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total rat heart + streptococci (1+2)	93	46	26	16	11	36	15	11	5	18	5	10	5	11	7
Total rat connective tissue + streptococci (4+5+6)	75	39	29	21	15	25	16	17	9	15	12	13	9	8	7
Total rat heart and rat connective tissue + streptococci (1+2+4+5+6)	168	43	27	18	12	26	16	14	6	17	8	11	6	10	13
Total controls (11+12+13+14+15+16)	148	4	1	3	3	5	1	5	3	1	1	0	0	2	1
Controls, exclusive of rat kidney + streptococci	91	3	1	2	2	1	0	0	0	0	0	0	0	2	0
Controls, treated with tissue or streptococci alone	46	2	0	2	2	2	0	0	0	0	0	0	0	2	0

1+ = slight changes; 2+ = well developed lesions (see page 9).

24

heart or the various connective tissue antigens and streptococci. Connective tissue is the only component common to all of the tissue antigens which were effective in inciting cardiac lesions. The available evidence therefore suggests that the site of the antigen which incites production of the autoantibodies responsible for these cardiac lesions is the connective tissue.

COMMENT

As described in the first article of this series,[1] autoantibodies to heart and connective tissue are produced in rats immunized with the homologous tissues in conjunction with killed streptococci. The data presented in this paper indicate that the cardiac lesions described are the result of the reaction occurring between these autoantibodies and the corresponding tissues in vivo. Presumably a number of different autoantibodies are formed as a response to injections of materials as crude as mixtures of streptococci and emulsion of heart, connective tissue or skeletal muscle. Evaluation of the changes observed in the various experimental groups, however, suggests that among these the autoantibodies to connective tissue are the ones that are responsible for the cardiac lesions observed, while autoantibodies to skeletal muscle, for instance, apparently have no capacity of pathogenic action. It seems, therefore, that the connective tissue is the site of that antigen which is of greatest interest with respect to the lesions produced.

As compared with analogous experiments concerning autoantibodies to kidney, reported previously,[3] the studies presented here have met with considerably greater difficulties. This was largely due to the fact that no clinical method of estimating the incidence and the intensity of these cardiac lesions was available. Thus, it was not possible to select the animals showing the most pronounced response and to study possibilities of intensifying the lesions by further immunizations in such a selected group. The problem of determining the exact time of onset in relation to the immunization schedule, as well as the life cycle of the lesions, was, for the same reason, rendered rather difficult. On the other hand, the serologic reactions of the autoantibodies to heart, skeletal muscle and connective tissue were, on the whole, considerably stronger than those to kidney reported previously.

It must also be pointed out that not all of the various batches of any one antigen, although they were prepared exactly in the same manner, were equally effective in producing cardiac lesions, a fact which had also been observed in our previous experiments with autoantibodies to kidney. There must, therefore, be a number of unknown factors which greatly influence the immunologic properties of the materials employed.

3. Cavelti, P. A., and Cavelti, E. S.: Arch. Path. **39**:148, 1945; **40**:158 and 163, 1945.

The cardiac changes described were not widespread or severe except in a few cases and had thus to be searched for by study of a number of histologic sections of each heart. Because of the smallness of the object, macroscopic inspection failed to yield reliable results, and, therefore, selection of the site of sectioning according to gross findings was not possible. Nevertheless, significant results seem to have been obtained, as shown by comparison of the changes found in the various groups of animals treated with different antigenic preparations.

The experimental glomerulonephritis produced by means of autoantibodies to kidney was, in many cases, of a progressive nature, the renal damage increasing over a period of many months without further injections being made in the animals. In the present studies, with regard to the cardiac lesions, there is no definite evidence of such progressivity except perhaps under conditions of repeated injections of effective preparations of streptococcus-tissue antigens.

As to the question as to what extent the lesions resemble human rheumatic lesions, I do not feel competent to make a definite statement. It can hardly be claimed that typical rheumatic fever has been produced in these experiments. In the cases of more pronounced reaction the structure of the lesions does, however, seem to resemble that seen in cases of rheumatic fever to some degree. More generally speaking, the changes produced are similar to the rheumatic cardiac changes in that they are located in all connective tissue structures of the heart and in that there is a great predominance of valvular lesions. Some degree of fibrotic thickening of the valves as a result of these lesions was seen relatively frequently. However, whether typical valvular heart disease with progressive changes resulting in valvular insufficiency or stenosis and subsequent cardiac decompensation has been produced could not be ascertained and was not obvious. It must further be borne in mind that the heart of a small laboratory animal such as the rat does not contain any large accumulation of connective tissue except perhaps in the valves. The interstitial and perivascular connective tissue of the myocardium is extremely scanty, a fact which might account, perhaps, for the relative infrequency and mildness of the lesions of these sites.

The animals were kept in small cages and in a room of relatively high temperature. Factors such as cold and body activity (fatigue) which are believed to play a role in the genesis of rheumatic fever were thus absent in these experiments.

The fact that changes affecting the valves and other connective tissue structures of the heart have been produced by means of a mechanism which could easily be conceived to operate in human rheumatic fever may be at least as significant as the question whether the experimental lesions are similar to those of rheumatic fever. On the basis of our

results. a working hypothesis of the genesis of human rheumatic fever could be formulated about as follows: During or succeeding the streptococcic infection which precedes a rheumatic attack by about two to three weeks an autogenous antigen is formed by a reaction in which streptococcic substances or products combine with components of the host's tissues. perhaps connective tissue. This antigen incites formation of specific antibodies. which in turn precipitate the rheumatic lesions by reacting in vivo with the antigen situated in the tissues.

From our in vitro studies it would seem that once the formation of autoantibodies has been incited, the streptococcic component is no longer necessary for the ensuing action of these antibodies on the tissues, as our experimental autoantibodies reacted in vitro with the plain tissue component, i.e., in absence of streptococcic material. The hypothesis presented could easily explain the well known and peculiar relationship between streptococcic infection and rheumatic fever, which seems to be an indirect one. The interval between the two would correspond to the time necessary for the formation of the autoantibodies, representing the pathogenic agent of rheumatic fever.

SUMMARY

Rats immunized with combinations of killed streptococci and rat heart or connective tissue form autoantibodies to these tissues, demonstrable in vitro, and, apparently as a result of the pathogenic action of these autoantibodies, undergo changes affecting chiefly the valves and the other connective tissue structures of the heart. In a broad sense these changes may be considered to resemble those of rheumatic fever.

AN ANALYSIS OF CASES OF RADIAL HEMIMELIA

RONAN O'RAHILLY, M.Sc., M.B., B.Ch.

NEWCASTLE-UPON-TYNE, ENGLAND

PEROMELIA

A N EXAMINATION of congenital anomalies of the upper limb suggests that many of the minor deficiencies occurring in the carpal or the digital regions represent a less widespread degree of a more serious longitudinal or ray defect, as distinct from a segmental or annular deformity. Such anomalies (fig. 1), grouped broadly under the heading of peromelia (πηρός, maimed; μέλος, limb), include total absence of the limb (amelia or ectromelia), absence of the forearm and hand (hemimelia), absence of the hand (acheiria or ectrocheiria) and absence of a digit (adactylia or ectrodactylia). In hemimelia the ulnar part of the limb may be intact (radial hemimelia) or less frequently the radial portion may persist (ulnar hemimelia). Furthermore, a middle part may be absent while those portions proximal and distal to it are present, an extreme example of this intercalary peromelia being presence of the shoulder and the hand with absence of the arm and forearm (phocomelia).

RADIAL HEMIMELIA

Radial hemimelia, according to Bunnell,[1] is a deficiency "of the radial bud and, therefore, often includes the carpal and digital rays of the radius and the radial part of the end of the humerus, or the arrangement may be segmental of C.5 and C.6." The thumb is usually absent. In some cases the index finger is involved, because "the middle ray [that of the index] may be involved in abnormalities of either the radial or the ulnar rays" (Forbes[2]) or, according to Bunnell,[1] because the index is included in both the radial and the ulnar buds. Rarely the ulna is duplicated (for example, see Santero[3]). The soft tissues (muscles, arteries and nerves) are commonly affected, and a marked degree of talipomanus valga is usual. The deformity is relatively frequent; Kato[4] collected 253 cases from the literature up to 1923, and since that date

1. Bunnell, S.: Surgery of Hand, Philadelphia, J. B. Lippincott Company, 1944.

2. Forbes, G.: Anat. Rec. **71**:181, 1938.

3. Santero, N.: Arch. ital. di chir. **43**:173, 1936.

4. Kato, K.: J. Bone & Joint Surg. **6**:589, 1924.

many more instances have been described. It is often hereditary and bilateral, and may be associated with other deformities. Function is considerably impaired, and various types of corrective operations have been devised, one of the most popular being Albee's [5] method of splinting by means of a bone graft.

Pathologic Anatomy.—There is rarely oppportunity of investigating the disposition of the soft tissues in cases of radial hemimelia, and attention is directed chiefly to the bones, both because they may be investigated roentgenographically with ease and because their condition forms the basis for surgical amelioration. It seems somewhat surprising at first sight, therefore, that although an analysis of the osseous arrangement has been presented by Kato [4] and by Kanavel [6] the carpus should
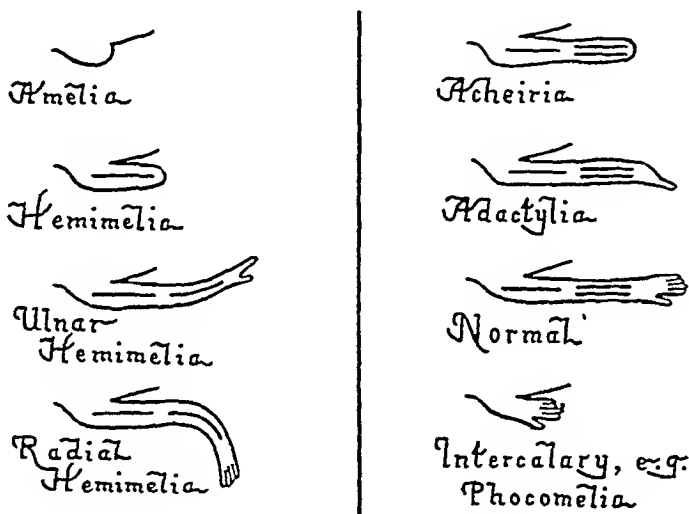
Fig. 1.—Classification of peromelia.

have received inadequate mention. The reason for this is that while the condition of the carpus of the adult can be determined from roentgenograms, in the great majority of the cases of radial hemimelia which have been described the anomaly occurred in an infant less than 1 year old and the disposition of the carpalia is unknown except in those few instances in which dissection was possible.

With a view to elaborating the pathologic anatomy of radial hemimelia and at the same time determining the components of the radial ray of the human upper limb I investigated in the literature 42 limbs in which the radius (or at least its distal part) was absent and in which the carpus was either described or illustrated as a consequence of having been examined by dissection or roentgenography. Two of these 42 limbs

5. Albee, F. H.: Ann. Surg. **87**:105, 1928.

6. Kanavel, A. B.: Arch. Surg. **25**:1 and 282, 1932.

Forty Cases of Radial Hemimelia in Which the Distal Part of the Radius Was Absent

(a, absent; r, reduced; p, present)

		Scaphoid Bone	Trapezium	First Metacarpal Bone	Phalanges of Thumb	Lunate Bone	Trapezoid Bone
Albee [5]	1928	a	a	a	a	p	p
		a	a	a	a	p	p
Bergerhoff, W.: Forschr. a. d. Geb. d. Röntgenstrahlen **36**: 376, 1927................	1927	a	a	r	p	p	p
		a	a	(p)	p	p	p
Bertwistle, A. P.: Brit. M. J. **1**: 325, 1923......	1923	a	a	a	a	p	p
	1923	a	a	a	a	p	p
Davidson, A. J., and Horwitz, M. T.: J. Bone & Joint Surg. **21**: 462, 1939................	1939	a	a	a	a	?	p
Drinnenberg, A.: Ztschr. f. orthop. Chir. **63**: 297, 1935	1935	a	a	a	a	p	p
Fairbank, H. A. T.: Brit. J. Surg. **1**:553, 1913	1913	?p	?p	a	a	?	?
Fontes, V.: Arq. de anat. **13**: 191, 1929-1930...	1930	a	r	r	r	p	r
Forbes [2]	1938	a	a	a	a	p	a
de Freitas Pereira, M. J., and de Lima, J. A. P.: Arq. de anat. **13**: 35, 1929-1930..	1930	a	a	a	a	?a	a
Grant, D. N. W.: Mil. Surgeon **69**: 518, 1931..	1930	a	a	a	a	p	p
Grant, J. W. G.: Brit. J. Surg. **18**: 166, 1930..	1931	a	a	a	a	p	p
Gruber, W.: Virchows Arch. f. path. Anat. **40**: 427, 1867	1867	r	a	a	a	p	p
Haas (Bunnell [1])	1944	a	a	r	r	p	r
Hill, L. L., Jr.: Surg., Gynec. & Obst. **65**:475, 1937	1937	p	p	p	p	p	p
		p	p	p	p	p	p
Jones, H. W.: J. Anat. **70**: 146, 1926.........	1926	a	a	a	a	p	p
McWhirter, R. (unpublished)..................	....	a	a	a	a	p	p
Meckel (Gruber)	1867	a	a	a	a	?	?
		a	a	a	a	?	?
Milne, J. A.: Brit. M. J. **2**: 821, 1915.........	1915	p	p	p	p	p	p
Schekter, L.: Gaz. d. hôp. **106**: 913, 1933......	1933	a	a	a	a	p	p
Silvester (Gruber)	1867	a	a	a	a	?	?
Stoffel, A., and Stempel, E.: Ztschr. f. orthop. Chir. **23**: 1, 1909..........................	1909	a	r	a	r	p	p
	1909	a	a	a	r	p	p
	1909	a	a	a	a	p	p
	1909	a	a	a	a	p	p
	1909	a	a	a	a	p	p
		a	a	a	a	r	p
	1909	a	a	a	a	p	a
		a	a	a	a	p	p
	1909	a	a	a	a	p	p
Tubby, A. H.: Deformities, Including Diseases of Bones and Joints, New York, The Macmillan Company, 1912. See Weigel.							
Tyrie: J. Anat. **28**: 411, 1894..................	1894	a	a	a	a	p	p
Tyrie: Ibid.	1894	p	p	p	p	p	p
		a	a	a	a	p	p
Tyrie	1894	p	p	p	p	p	p
Wakeley, C. P. G.: J. Anat. **65**: 506, 1931....	1931	a	a	a	a	a	p
Weigel (Tubby)	1912	a	a	a	a	a	p
Windle, B. C. A.: J. Anat. **36**: 306, 1902......	1902	p	p	a	a	a	a

had only one finger each, together with one and no carpale respectively. Out of the remaining 40 the following conditions were collected:

Scaphoid bone	absent in 33 and reduced in 1
Trapezium	absent in 32 and reduced in 2
First metacarpal bone	absent in 32 and reduced in 3
Phalanges of thumb	absent in 30 and reduced in 5

It can be seen, therefore, that each of the bones named may be present in certain cases in which the radius is absent; they are usually missing, however, and were all absent simultaneously in 27 of the 40 cases.
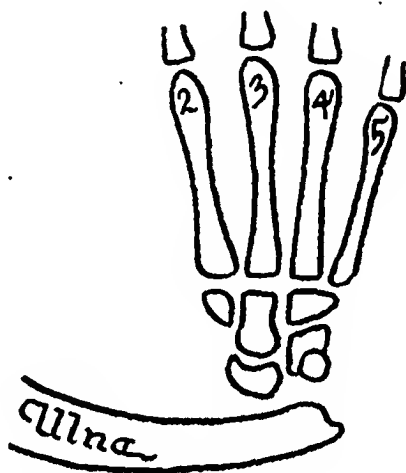
Fig. 2.—Typical carpus in radial hemimelia. For description, see text.

Per contra, a similar investigation of the nearby carpalia in cases of radial hemimelia revealed the following conditions:

Lunate bone	absent in 3 of 35 cases
Trapezoid bone	absent in 4 of 36 cases

Both the lunate and the trapezoid bone were present simultaneously in 30 of 36 cases.

Figure 2 shows the typical condition of the carpus in radial hemimelia; in 17 of the 40 cases the carpus conformed to this representation. The figures listed to this point are illustrated diagrammatically in figure 3, and the individual cases are given in the accompanying table. Sex, age and side of body were recorded too infrequently to be included. If cases of bilateral radial hemimelia had been inserted as cases of unilateral hemimelia 9 cases would have been deducted from the list; this has not been done, however, as the arrangement is by no means necessarily the same on both sides.

There are cases in which the more distal part of the radial ray alone is affected; in 13 such cases the radial styloid process was present

in 2 and absent in 6; in 5 its presence or absence was unrecorded. In the majority of these cases the scaphoid bone, the trapezium and the first metacarpal bone were either absent or reduced, and the phalanges of the thumb were sometimes absent also.

ULNAR HEMIMELIA

It has not been possible to investigate ulnar hemimelia and the constitution of an ulnar ray or rays in a manner similar to that in

■ = absent; ▨ = reduced
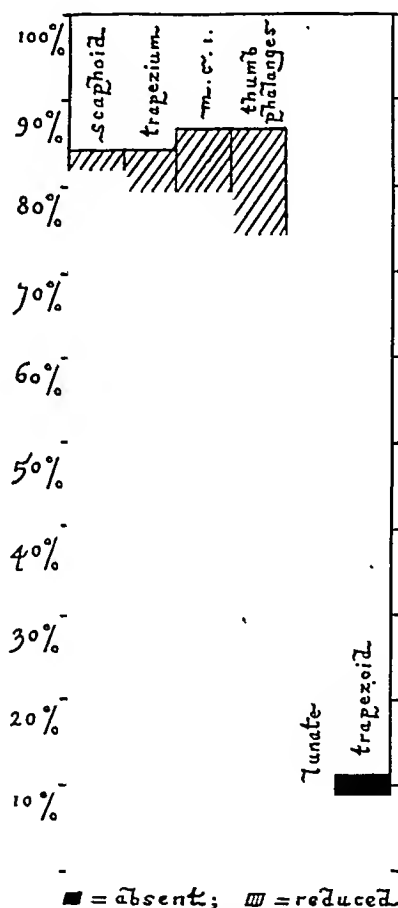
Fig. 3.—Graphic representation of carpalia in 39 cases of radial hemimelia. (This illustration reached the printers before McWhirter's case had been seen.) *M. C. 1* signifies first metacarpal bone.

which the radial deficiency was reviewed, because the condition is rare and because the carpus is more involved, rendering the individual bones which develop more difficult to identify. Some of these cases have been reviewed by Kanavel.[6] Sometimes the entire carpus is absent, but the thumb and usually the index finger are present.

FUNCTIONAL IMPLICATIONS AND ETIOLOGIC CONSIDERATIONS

The applications of these investigations to the functional anatomy of the carpus and the etiology of peromelia have been discussed elsewhere (O'Rahilly [7]).

SUMMARY

A general classification of ray defects of the upper limb is presented.

The osseous anatomy of radial hemimelia is described and the typical condition illustrated. An analysis of recorded cases revealed that in the majority of instances the scaphoid bone, the trapezium, the first metacarpal bone and the phalanges of the thumb are absent while the other skeletal elements of the hand are present. This series of usually interdependent bones constitutes a radial ray.

The composition of the ulnar ray or rays is more difficult to determine, and it has not proved practicable to survey the pathologic anatomy of ulnar hemimelia.

---

7. O'Rahilly, R.: J. Anat. **80**:179, 1946.

POLYCYSTIC DISEASE OF THE KIDNEY

A Review

. P. P. LAMBERT, M.D.*

BRUSSELS, BELGIUM

BECAUSE of the mystery that still surrounds its genesis, polycystic disease of the kidneys continues to evoke the curiosity of both pathologist and clinician. It is a disease of paradox, appearing typically either in the newborn or as an affliction of adult life. In the first case it usually leads to the death of the infant without delay, while in the adult it is found to be compatible with a long period of survival, death usually occurring in the second half of life. In either instance the kidneys may be enlarged, in the newborn so much so as to result in dystocia; or, on the contrary, they may be smaller than the normal.

Other kinds of congenital disarrangement are often observed in the infant with cystic kidneys, such as polydactyly, anencephaly or meningocele, and still more important, associated congenital defects of the urinary tract with resulting aplasia of the calices and pelvis, stenosis of the ureters or occlusion of the urethra. Because of this frequent association, the congenital defects of the extrarenal excretory passages have indeed been considered a possible cause of the cyst formation of the kidney and the disease interpreted as a sort of hydronephrosis (Wigand[1]; Marchand[2]). Although from the clinical standpoint it may indeed at times be difficult to differentiate between congenital hydronephrosis and polycystic disease in the newborn, in the adult the distinction can be easily made from the pathologic as well as from the clinical point of view (Bell[3]).

Adult patients with polycystic kidneys usually remain without serious trouble for many years, complaining only of mild lumbar pains that may be aggravated by hard physical work. At times, however, the pains become so unbearable as to suggest renal stone or hydronephrosis and

---

1. Wigand, F.: Ueber congenitale Cystennieren (Cystenbildung durch Retention und cystische Degeneration), Inaug. Dissert., Marburg, R. Friedrich, 1899.

2. Marchand, F.: Verhandl. d. deutsch. path. Gesellsch. **2**:187, 1899.

3. Bell, E. T.: Am. J. Path. **11**:373, 1935.

are followed by the voiding of dark brown, blood-stained urine, an episode which is explained by rupture of a cyst wall and opening of the cyst into the pelvis. After many years signs of renal failure appear as they do in progressive Bright's disease, the most important being arteriosclerosis, hypertension, cardiac enlargement, mild retention of nitrogen and sometimes ocular symptoms. A remarkable fact is that in many cases albuminuria is absent. Palpation of bilateral lumbar tumors discloses the nature of the disease, though it should be kept in mind that the left kidney, although enlarged, may not be felt. In these cases pyelography may be useful.

Death usually occurs after a long period of progressive renal failure, but life may be shortened by the rupture of a vessel of the brain or of the meninges. This happened four times in the small series of 7 cases which my associates and I have observed and seems to be linked to small congenital aneurysms of the cerebral arteries (Snapper and Forminje [4]; Crowley and Martland [5]).

The macroscopic features of the polycystic kidney of the adult are so well known as to need no description here; however, it should be remembered that between the typical polycystic form and the so-called solitary cyst of the kidney many intermediate stages may be observed. To diagnose these lesser types of cystic renal disease during the life of the patient is difficult, and so these forms often appear as postmortem discoveries.

Since the macroscopic appearance of polycystic disease is usually so different in the adult and in the newborn, it is not surprising that certain investigators have abandoned the idea that it has the same genesis in both. However, a microscopic study of the condition in both has convinced most that the two forms are best considered as two different stages of a single disease.

MORPHOLOGY OF THE POLYCYSTIC DISEASE

Before summarizing the results of the observations of my colleagues and me, it is necessary to recall what has been contributed to this subject over past years and to examine the different hypotheses that previous workers have offered in explanation of the origin of the cysts.

FACTUAL OBSERVATIONS OF THE PAST

In the case of the newborn it may be easily seen that the majority of the cysts are distended nephrons or enlarged portions of nephrons which are separated from their excretory tubules (Beckmann [6]; Herx-

4. Snapper, J., and Forminje, P.: Acta med. Scandinav. **101**:105, 1939.

5. Crowley, O., and Martland, H., cited by Snapper and Forminje.[4]

6. Beckmann, O.: Virchows Arch. f. path. Anat. **9**:221, 1856.
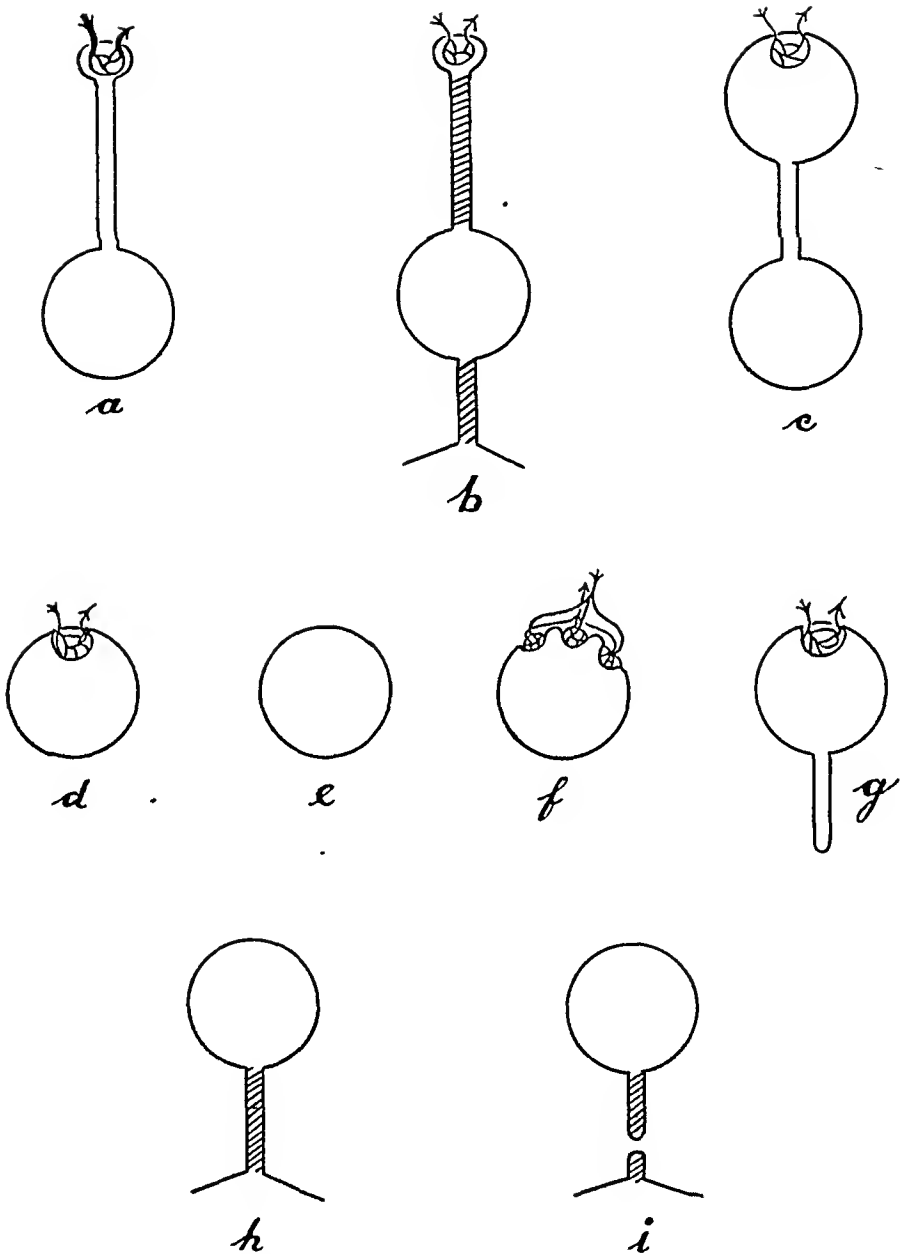
Fig. 1.—Various types of cystic nephrons previously described by other authors:
(a) Terminal cyst in the course of a closed nephron. (b) Cyst described by von
Mutach.[11] (c) Bicystic nephron. (d) Glomerular cyst. (e) Cystic vesicle. (f)
Glomerular cyst with several glomerular tufts. (g) Glomerular cyst with blind
tubule. (h) Excretory cyst opening into the calix. (i) Closed excretory cyst.

heimer [7]; Witte [8]; Busse [9]; Berner [10]; von Mutach [11]). In the case of the adult, however, technical difficulties encountered in the reconstruction of the nephrons have prevented in the past a thorough investigation of the topography of the distorted structures.

A brief recapitulation of the various types of cystic nephrons that have been observed in the polycystic kidney of the newborn by previous authors is shown in figure 1.

One type of cyst, first described by Beckmann [6] in 1856, is the so-called glomerular cyst. The glomerular cyst is a closed cavity lined by flattened epithelium. Sometimes a tubule appears to leave the cyst, but when followed in serial sections it is found either to end blindly or to dilate into a terminal sac (Dunger [12]; fig. 1 *c, d* and *g*). The vascular tuft of the glomerular cyst is usually well formed but occasionally is poorly differentiated (Busse [9]). Sometimes several tufts of capillaries appear in one cyst, the apparent result of the traction that is made on the original tuft during expansion of the cyst (fig. 1 *f*).

Near the glomerular cysts, tubular cysts may be observed. In many instances the tubular cyst appears to be developed in the distal part of a closed nephron (fig. 1 *a*). Serial sections have shown the tubule to be short and straight, opening at one end into the glomerulus and at the other into the cyst, which is always unipolar. At times the cyst is placed in the middle part of the nephron (fig. 1 *b*), where it may be either unipolar or bipolar: if it is unipolar, the dilatation occurs laterally in the tubule wall, but if it is bipolar, the proximal part of the tubule forms one pole and the distal part forms the other. The further course of this distal portion has been rarely followed carefully. In a case reported by von Mutach [11] it was followed to its opening in the calix, but in this instance the situation was complicated by aplasia of both ureters. We could not find in the literature another report in which cystic nephrons are described as opening into the pelvis, a point the importance of which will appear in the description of our personal observations.

A third type of cyst, described by Ribbert,[13] is the so-called excretory cyst. Cysts of this type usually open into the pelvis, and Berner,[10] by injecting carmine-gelatin into the proximal part of the ureter, demon-

7. Herxheimer, G.: Virchows Arch. f. path. Anat. **185**:52, 1906.

8. Witte, P.: Erworbenes multiloculares Adenokystom und angeborene cystische Entärtung der Nieren, Inaug. Dissert., Königsberg i. Pr., E. Rautenberg, 1896.

9. Busse, O. U.: Virchows Arch. f. path. Anat. **175**:442, 1904.

10. Berner, O.: Die Cystenniere, Jena, G. Fischer, 1913.

11. von Mutach, A.: Virchows Arch. f. path. Anat. **142**:46, 1895.

12. Dunger, R.: Beitr. z. path. Anat. u. z. allg. Path. **35**:445, 1904.

13. Ribbert, H., in Born, G., and others: Bibliotheca medica, Cassel. T. G. Fisher & Co., 1896, pt. C, no. 4.

strated dye in many of them (fig. 1 *h*). A smaller number could not, however, be delineated in this way and were therefore considered by him to be isolated from the pelvis (fig. 1 *i*). The collecting tubules are frequently pinched and distorted by proliferation of connective tissue, which may be abundant in the papillae of polycystic kidneys (Ruckert [14]; Aschoff [15]; Dettmer [16]). And lastly, aglomerular vesicles without any tubular connection are occasionally met in the cortex of the polycystic kidney of the newborn (fig. 1 *e*).

Past investigators have emphasized the abundance of connective tissue that is present in the polycystic kidneys of the newborn. Histologically, it is normal embryonic mesenchyme, which becomes denser around the cysts and is sometimes mixed with muscle fibers (Busse [9]). They have also pointed out that scattered among "round cells" resembling lymphocytes, true isolated epithelial cells can be seen (Busse [9]). Sometimes these are grouped to form small vesicles. Mesenchymatous connective tissue, muscle fibers and isolated epithelial cells are considered to be embryologic remnants because they are normally observed in earlier stages of fetal life.

In rare instances, blood vessels have been said to take part in the cystic process (Berner [10]), a condition which has also been described by Grafflin [17] as occurring normally in a fish kidney. Dyckerhoff [18] expressed the opinion that certain renal cysts have a lymphatic origin since they differ from others by the homogeneous character of their albuminous content and by the absence of luminal casts and epithelial fragments.

Theoretic Conclusions from These Observations.—The most important structural feature of the polycystic kidney of the newborn is that the cystic nephrons are closed systems, not communicating with the pelvis. The only exception, observed in the case of von Mutach,[11] concerns a kidney with ureteral aplasia. For this reason it has been generally agreed that tubular obstruction has something to do with the cystic involvement. This was stated by Virchow [19] as long ago as 1855, who first suggested that tubular casts or insoluble calcium salts might be sufficient to obstruct the urinary flow and so lead to cystic dilatation. Later, however, in 1890, he attributed the formation of

14. Ruckert, A., in Festschrift für Prof. Orth., Berlin, A. Hirschwald, 1903, p. 475.

15. Aschoff, L.: Verhandl. d. deutsch. path. Gesellsch. **7**:69, 1904.

16. Dettmer, H., in Festschrift für Prof. Orth., Berlin, A. Hirschwald, 1903, p. 554.

17. Grafflin, A. W.: Biol. Bull. **72**:247, 1937.

18. Dyckerhoff, K. H.: Virchows Arch. f. path. Anat. **216**:116, 1919.

19. Virchow, R.: Gesammelte Abhandlungen zur wissenschaftlichen Medicin, Frankfurt a. M., Meidinger Sohn u. Comp., 1856.

the cysts to fibrous tissue occlusion of the excretory tubules, which he thought resulted from prenatal inflammation.[20]

This explanation was attacked by Hanau,[21] who held that the cystic transformation of the nephron resulted from congenital aplasia of the medullary tubules. This idea received wider support some years later when embryologists (Schreiner [22]; Felix [23]) showed that the renal tubules develop as the result of a union of two embryologic elements, one of these, the metanephric, forming the glomerulus and the tubule of the nephron, while the other, the ureteric, provided by its dichomatous division the collecting tubules of the medulla. Various processes might conceivably produce a failure of union between the primitive nephrons and the collecting tubules; on account of the frequency of congenital defects of the extrarenal excretory ducts of the polycystic kidney of the newborn, many authors have suggested that aplasia of the intrarenal excretory (collecting) tubules might be the most probable cause of disunion of the two embryologic structures (Dettmer [16]; Ruckert [14]).

According to Busse [9] and Berner,[10] arrested development of the metanephrons results in faulty union of the two embryologic parts of the kidney; in their opinion this conclusion is borne out by the finding of many embryologic remnants in the polycystic kidney of the newborn. More recently McKenna and Kampmeier [24] have given another and somewhat similar explanation of the formation of the cysts. During fetal life, the ureters divide dichotomously to form excretory ducts, which rapidly become attached to nephrons of metanephric origin. However the excretory ducts of the first and second order separate from their nephrons to form the calices, and the nephrons isolated by this process usually atrophy and disappear. The authors suggest that some may persist and become cystic. This suggestion might explain the formation of a few cysts in the central part of the kidney but not the origin of the widely scattered and numerous cysts of polycystic disease.[25]

20. Virchow, R.: Deutsche med. Wchnschr. **36**:11, 1892.

21. Hanau, L.: Ueber congenitale Cystennieren, Inaug. Dissert., Giessen, C. von Münchow, 1890.

22. Schreiner, K.: Ztschr. f. wissensch. Zool. **71**:1, 1902.

23. Felix, W., in Keibel, F., and Mall, F. P.: Handbuch der Entwicklungsgeschichte des Menschen, S. Hirzél, Leipzig, 1910.

24. McKenna, J. M., and Kampmeier, O. F.: J. Urol. **72**:37, 1934.

25. Since this was written, an article by Norris and Tyson (Am. J. Path. **23**:201, 1947) has called my attention to a publication of theirs which had not reached me during the war years (J. Urol. **46**:147, 1941) in which, after studying serial sections and wax reconstructions of the collecting ducts of polycystic kidneys in 4 cases, they explain the origin of renal cysts by an extension of the normal process of degeneration noted by Kampmeier in the first generation of nephrons to include some or all of the later generations.

Finally must be mentioned the theory, rejected now by most modern investigators, that the formation of cysts is of the nature of a neoplastic process (Brigidi and Severi [26] ; Chotinsky [27] ; Nauwerck and Hufschmied [28]).

PERSONAL OBSERVATIONS

The personal investigations [29] to be summarized now have been directed toward the solution of the two fundamental problems of the pathogenesis, namely: What structural changes have occurred in the nephrons of the polycystic kidney and what are the functional capabilities of these altered nephrons?

The structural changes were examined by comparing the cysts in the polycystic kidney of the adult with those in the polycystic kidney of the newborn.[29c] Since it is the continuity of the nephron and cysts that is significant, these structures were graphically reconstructed by the method of Peter,[30] and the results of the observations thus made were compared with the findings of previous workers.

The functional capabilities of the nephrons which are so profoundly altered in structure have been examined by determining the composition of the fluid which may be collected from the cysts and also by observing the amounts of a foreign material appearing in the cyst fluid after the material had been injected into the blood.[29 b,d] Inulin was chosen as the test substance because, as shown by Smith,[31] it is excreted only by the glomeruli.

The physiologic examination of the functional capacity of the nephrons of the polycystic kidney as well as the morphologic study of their structure has convinced us that in many instances nephrons with extreme cystic dilatation of their tubules maintain much of the physiologic activity of the normal nephron and that they contribute urine to the excretory process. This conclusion would afford an adequate explanation of the fact that in the polycystic kidney of the adult, at least, renal failure is a late consequence of the disease and

26. Brigidi, V., and Severi, A.: Sperimentale **46**:1, 1880.

27. Chotinsky, A.: Ueber Cystenniere, Inaug. Dissert., Bern, B. F. Haller, 1882.

28. Nauwerck, C., and Hufschmied, K.: Beitr. z. path. Anat. u. z. allg. Path. **12**:1, 1892.

29. (*a*) Lambert, P. P., and Cambier, P. P.: Beitr. z. path. Anat. u. z. allg. Path. **101**:470, 1938. (*b*) Lambert, P. P., and Muller, P.: Acta med. Scandinav. **101**:338, 1939. (*c*) Lambert P. P.: Le rein polykystique, Paris, Masson & Cie, 1943. (*d*) Muller, P., and Lambert, P. P.: Presse méd. **48**:186, 1940.

30. Peter, K.: Rekonstruktionsmethoden, in Abderhalden, E.: Handbuch der biologischen Arbeitsmethoden, Vienna, Urban & Schwarzenberg, 1921, vol. 9, p. 1.

31. Smith, H. W.: The Physiology of the Kidney, London, Oxford University Press, 1937.

probably linked to the secondary sclerosis that is found only on post-mortem examination.

Structural Alterations in the Nephrons of the Polycystic Kidney.—
I have had an opportunity to examine in graphic reconstructions 2 cases of polycystic disease of the newborn [32] and 5 cases of polycystic disease of the adult..
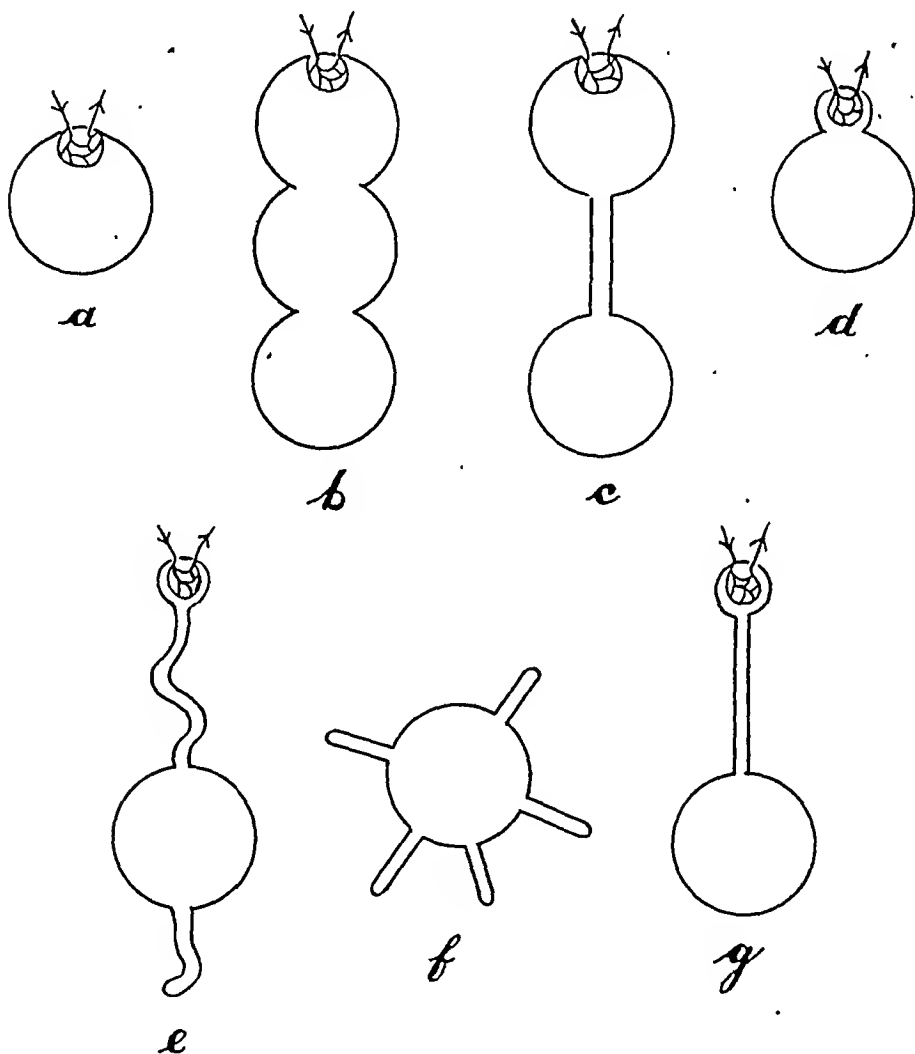
Fig. 2.—The various types of cysts observed in 2 cases of polycystic kidney of the newborn: (*a*) Closed glomerular cyst. (*b*) Closed nephron with several cysts. (*c*) Closed nephron with glomerular and terminal cysts. (*d*) Closed subglomerular cyst. (*e*) Bipolar tubular cyst in the course of a closed nephron. (*f*) Cystic vesicle with blind tubules. (*g*) Terminal cyst in a closed nephron.

(*a*) Polycystic Kidney of the Newborn: Since in the first case of polycystic kidney of the newborn the kidneys present the structural changes seen in most polycystic infantile kidneys, they are described in brief detail.

32. R. de Puysseleyr (J. d'urol. **41**:201, 1936) gave me permission to study this material.

The infant was born dead at 7½ months. Both kidneys were enlarged and so filled with small cysts, averaging 2 mm. in diameter, that on macroscopic examination no normal parenchyma could be seen. The papillae and calices were absent, although there was a narrow pelvis and a patent ureter.
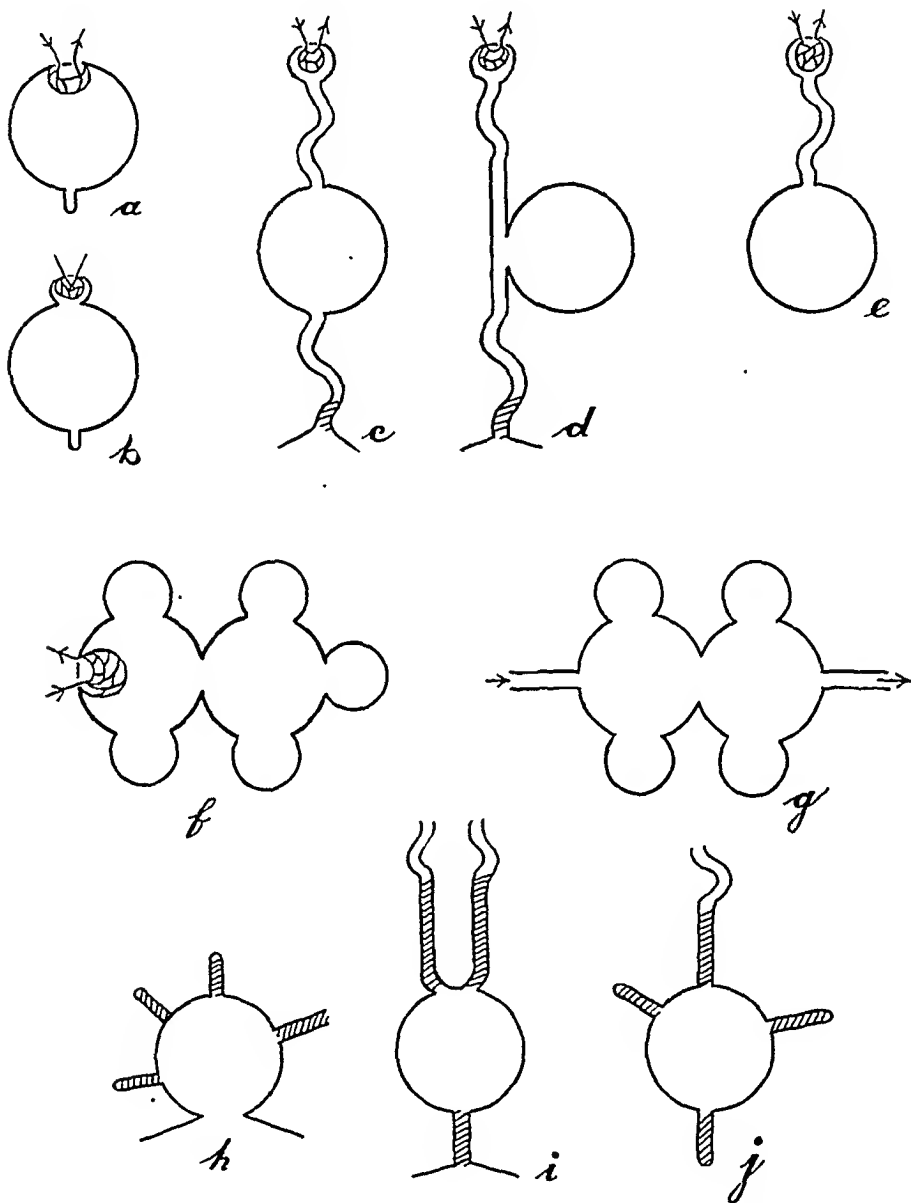
Fig. 3.—Various types of cyst seen in the polycystic kidney of the adult. Shaded portions indicate collecting tubules. (a) Glomerular cyst with blind tubule. (b) Subglomerular cyst. (c) Bipolar cyst in an open nephron. (d) Unipolar cyst in an open nephron. (e) Terminal cyst in a closed nephron. (f) Multilocular glomerular cyst. (g) Multilocular tubular cyst. (h) Cyst of the calix. (i) Tripolar excretory cyst. (j) Excretory cyst unconnected with the pelvis.

Microscopic sections showed two distinct layers, a thin external zone formed by the cystic nephrons and a much thicker internal zone formed by irregular cysts and the mesenchymal connective tissue interspersed between them.

The cysts in the external layer were found to be glomerular, subglomerular or tubular. None of them opened into the pelvis (fig. 2 *a* and *b*). The subglomerular type of cyst opened into an adjacent glomerular cavity but did not communicate with a tubule (fig. 2 *d*). The tubular type was either unipolar or bipolar. The unipolar cyst was situated in the distal (fig. 2 *g*) and the bipolar cyst in the middle portion of the nephron, generally at the point where the proximal and the distal convoluted tubule meet (fig. 2 *e*). Their glomerulus appeared normal, and the distal tubule in no case opened into a collecting tubule (fig. 4). Cystic nephrons of the tubular type presented the typical S-shaped form of the embryologic nephron in the second month of fetal life. In one of them (fig. 5) the whole nephron, including the glomerulus, was involved in the cystic change.

The cysts of the internal zone were closed, irregular vesicles Sometimes blunt short tubes were found to leave the cyst and to pursue a course between
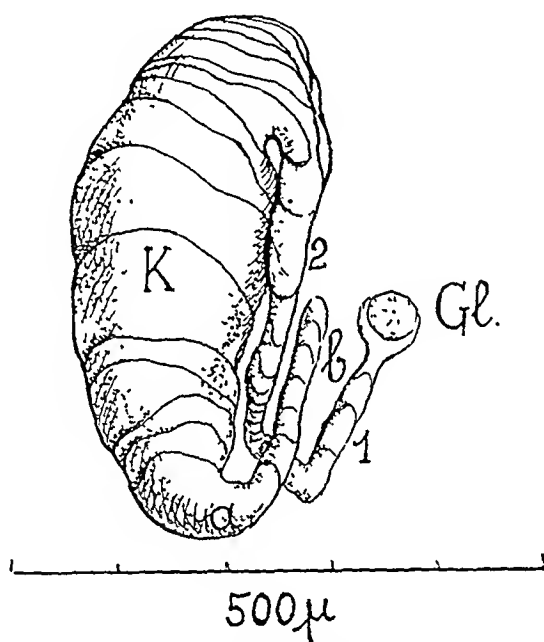
Fig. 4.—Bipolar tubular cyst in the course of a closed nephron. Note the typical S-shaped form of the closed nephron. *Gl* indicates the glomerulus.

neighboring cysts (fig. 2 *f*). What the origin of this type of cyst may be is unknown; it would appear that they are either cystic metanephric vesicles or dilated collecting tubules The former hypothesis seems the more likely, for if they were cysts of the collecting tubules, one might wonder why they never open into normal excretory ducts or into the pelvis.

Conclusions: The study of serial sections of the kidneys of a premature infant and that of a newborn infant and the preparation of graphic reconstructions of the cystic nephrons added little to what had been already established by previous investigators. The essential fact remains that the cystic nephrons are always separated from their excretory tubules and exist as closed vesicles. It does not follow from this fact that the cyst formation was of necessity the result of a failure

of fusion of the metanephric and ureteric components of the kidney, for it may well be that the cyst formation, taking place in early fetal life, is itself the cause of the disassociation between the two embryologic elements. It seems impossible to prove which of these two hypotheses is the more likely in the light of the 2 cases studied by me though the occurrence of glomerular cystic vesicles which cannot be explained by a failure of fusion of tubules, since no tubule is present, is suggestive that the latter view is more likely correct
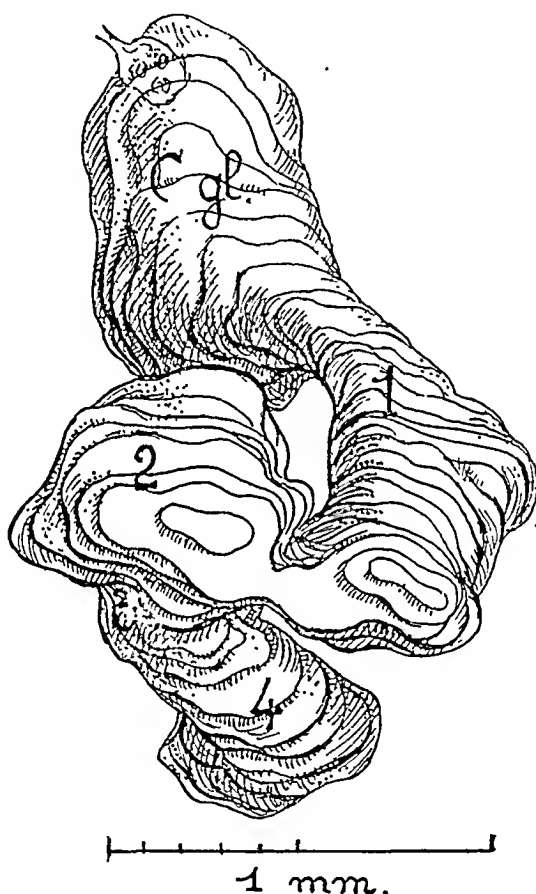
Fig. 5.—Closed polycystic nephron: *C gl* indicates a glomerular cyst; *1, 2, 3* and *4,* tubular cysts. The S-shaped form of the nephron is well shown.

That the failure of union of nephron and excretory tubule is not the decisive factor in cyst formation would be demonstrated if one could find cystic nephrons opening into excretory ducts and therefore able to excrete urine. The only example known in the kidney of the newborn is that described by von Mutach [11] but, as pointed out previously, on account of the bilateral atresia of the ureters, a mechanical factor in cyst formation cannot be excluded in his case.

With a view to following this problem further, the polycystic kidney of the adult will next be described.

(*b*) Polycystic Kidney of the Adult: Since the answer to our problem is concerned with the continuity of the cysts, the tubules and the renal pelvis, it is obvious that it will be answered only by a reconstruction of this continuity. Such a reconstruction of a cystic nephron begins with a careful examination of serial sections of a cyst wall until the opening of the tubule is found; the tubule must then be followed
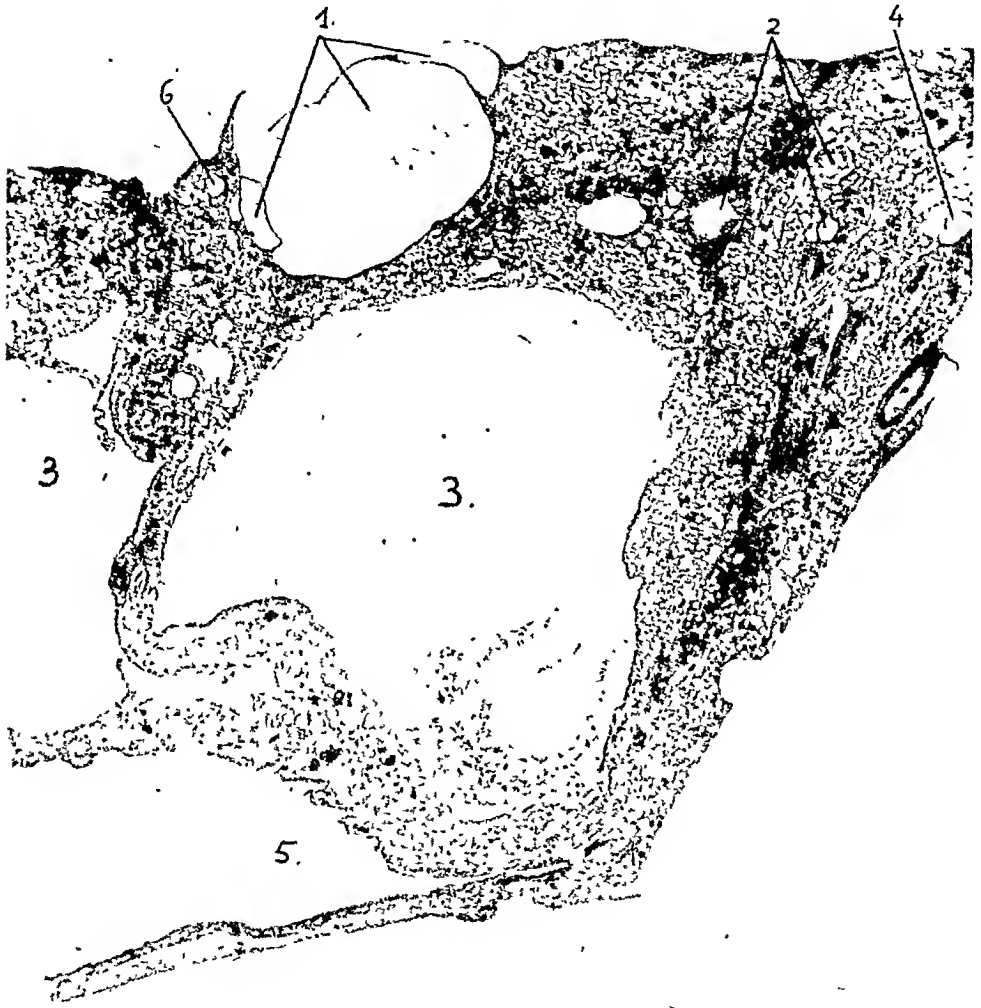
Fig. 6.—Low power microscopic view of the polycystic kidney of the adult: *1* indicates three glomerular cysts; *2*, tubular cysts formed in the course of Henle's loop; *3*, a voluminous excretory cyst; *4*, a multilocular cyst; *5*, part of a calix; *6*, a cyst of the distal convoluted tubule which is seen reconstructed in figure 8.

upward to the glomerulus and downward toward the collecting tubule. To produce a comprehensive picture of what he has seen, the investigator has then to create an objective representation of the cystic cavity and its associated tubules. The Born technic results in a wax model but is so arduous and time-consuming as to be impracticable if any number of nephrons are to be examined, so that in our study Peter's

graphic method was used, a procedure which shows the object in three-dimensional perspective.

A section through the adult polycystic kidney generally shows three types of cysts, which are distributed roughly in three zones (fig. 6).

1. Large, glomerular cysts are found immediately under the renal capsule. They generally contain a small amount of finely distributed granular albuminous material (fig. 6, *1*).

2. Smaller, tubular cysts are generally found at a deeper cortical level. They are as a rule filled with a dense homogeneous coagulum similar to that found in the distal tubules of patients with albuminuria (fig. 6, *2* and *6*).

3. In the medulla a third type, the excretory cyst, is found (fig. 6, *3*). It generally contains a heterogeneous precipitate composed of desquamated epithelial cells, casts and erythrocytes which are held in a fibrinous coagulum.

1. *Glomerular Cysts.* Twelve glomerular cysts were reconstructed, the diameter of the largest being 1.3 cm. The glomerular tufts were not dissimilar from those of normal glomeruli except that they were rather smaller. Their histologic structure was normal, and their capillaries were fully patent and contained unaltered erythrocytes, which suggests that an effective vascular supply had been maintained. In only 1 cyst was the glomerular tuft sclerosed. Openings in the cyst were occasionally found, but in subsequent serial sections these were found always to lead to a cul-de-sac and never to a patent proximal convoluted tubule (fig. 3 *a*). In 1 example the cyst appeared as a subglomerular closed cavity (fig. 3 *b*).

2. *Tubular Cysts.* Graphic reconstructions of 10 cysts of this type were made, and in 10 others the cyst walls and their connections were followed in serial sections without actual reconstruction. All of the 20 cysts communicated with the tubular system, a situation in striking contrast to that found in the study of glomerular cysts.

Two distinct types of tubular cysts were observed as follows:

Three of the 20 cysts studied were found to exist as termination of tubules of nephrons which started normally with glomeruli (fig. 3 *e*). In 2 of these instances the site of the cyst was in Henle's loop; in view of the extreme narrowness of the thin descending loop of Henle it is conceivable that even with the perfect series of sections at hand a continuation of the tubule distal from the cyst was missed.

All of the remaining 17 cysts were present in patent tubules; some were unipolar, and each of these appeared, therefore, as a dilatation of the side of a tubule (fig. 3 *d*); others were bipolar, and each of these produced a general ballooning of a tubule (fig. 3 *c*). In every case only one nephron opened into the cyst.
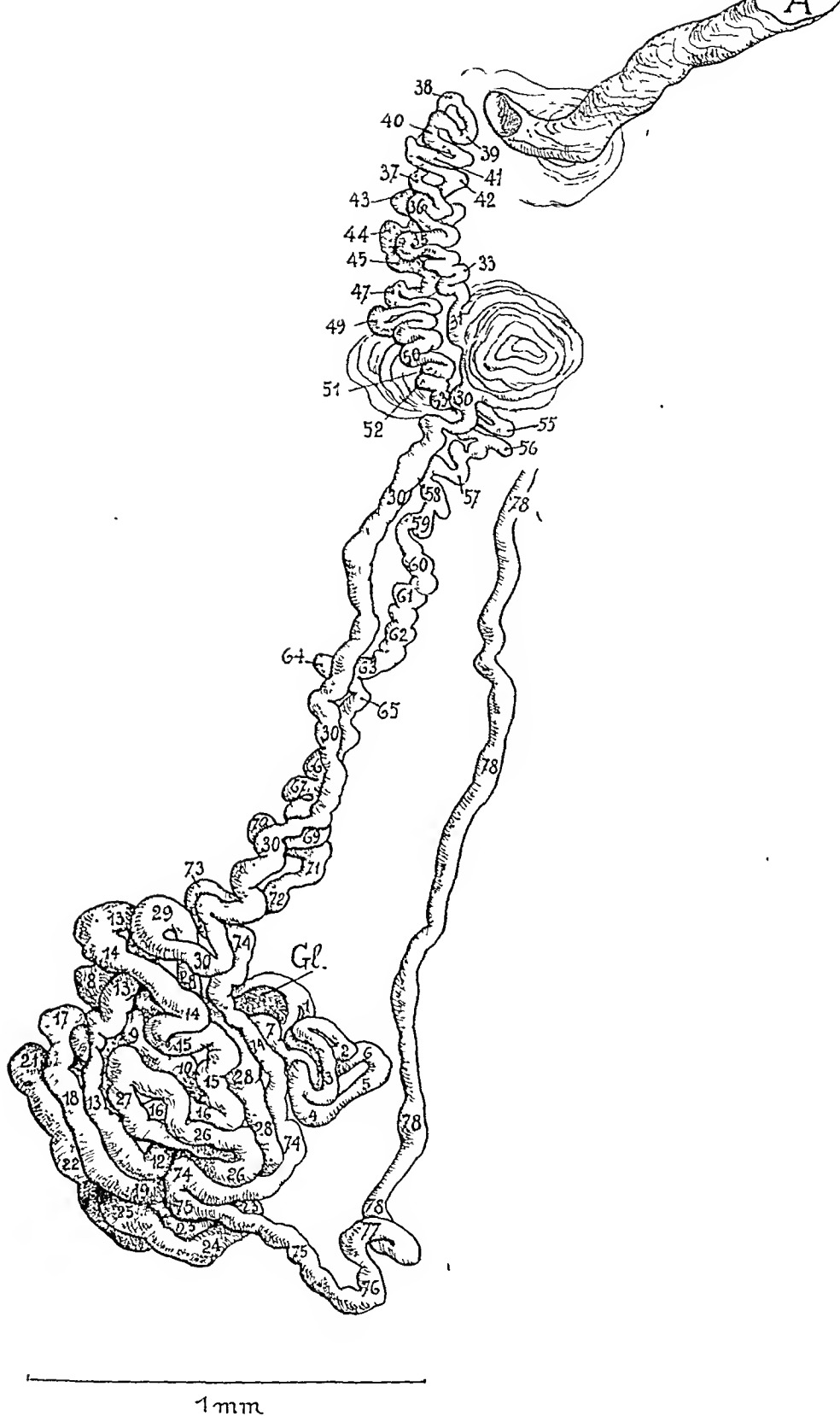
Fig. 7.—Graphic reconstruction of a complete open nephron containing a cyst, the bipolar cavity of which arises in the terminal straight portion of the proximal convoluted tubule (between *30* and *31*). The tubule is reconstructed as far as the collecting tubule (*76, 78*). *Gl* indicates the glomerulus of the nephron; *A*, a lobular artery.

Tubular cysts were observed in the proximal convoluted tubules, in the ascending and descending loops of Henle and in the distal convoluted tubules, and at least 1 cyst was situated at the junction of the distal convoluted tubule and the collecting tubule. Two examples of graphic reconstructions of these structures will illustrate the general findings.

Two cysts were observed in the course of a proximal convoluted tubule. One of them was completely reconstructed (fig. 7). The cyst was small and of the bipolar type and had developed at the junction of the terminal portion of the proximal convoluted tubule and the descending limb of Henle's loop, which in this particular case was tortuous on account of coexistent fibrous sclerosis. The glomerulus and the tubule appeared normal except that the epithelial cells of the first portion of the proximal convolution were flat and without an epithelial brush. No constriction was observed in the course of this nephron, which opened normally into the excretory tubule (7, 6 in fig. 7).

Two conclusions follow from this observation: first, that a failure of junction of the tubule of the nephron and its excretory component can be ruled out as a cause of the cystic involvement and, second, that it is also impossible to conclude, as Berner[10] did, that any arrest of development had occurred in this cystic nephron, since the different portions of it were everywhere well formed.

Moreover, the cause of the formation of cysts does not appear to be the result of mechanical back pressure imposed on the walls of the tubules. At least there was no apparent occlusion in the nephron as far as its union with the collecting tubule, though it must be admitted that one would have to continue the reconstruction of the latter to its ending in the calix to be sure that there was no obstruction at any point of the excretory channel.

This has in fact been accomplished in the case of a cystic nephron in which the cyst was situated in the terminal portion of the distal convoluted tubule. The nephron was examined from the glomerulus to the calix, but to simplify the drawing we have omitted the glomerulus, the promixal convoluted tubule and a part of Henle's loop. The nephron starts at 1 (fig. 8) just above the turn of Henle's loop, and from there on the more distal part of the nephron, the cyst (C) and the excretory tubules are shown in continuity.

In histologic section, abundant protein precipitate and cell débris were observed in the collecting tubule as well as in the calix at the point where the collecting tubule opens into it. At some points in the course of the tubule its lumen narrows, a result which was attributed to the proliferation of surrounding connective tissue, but the passage remains patent all the way to the calix. This observation strongly supports the argument that mechanical obstruction in the lower parts of the excretory tubules is not a factor producing the cyst formation
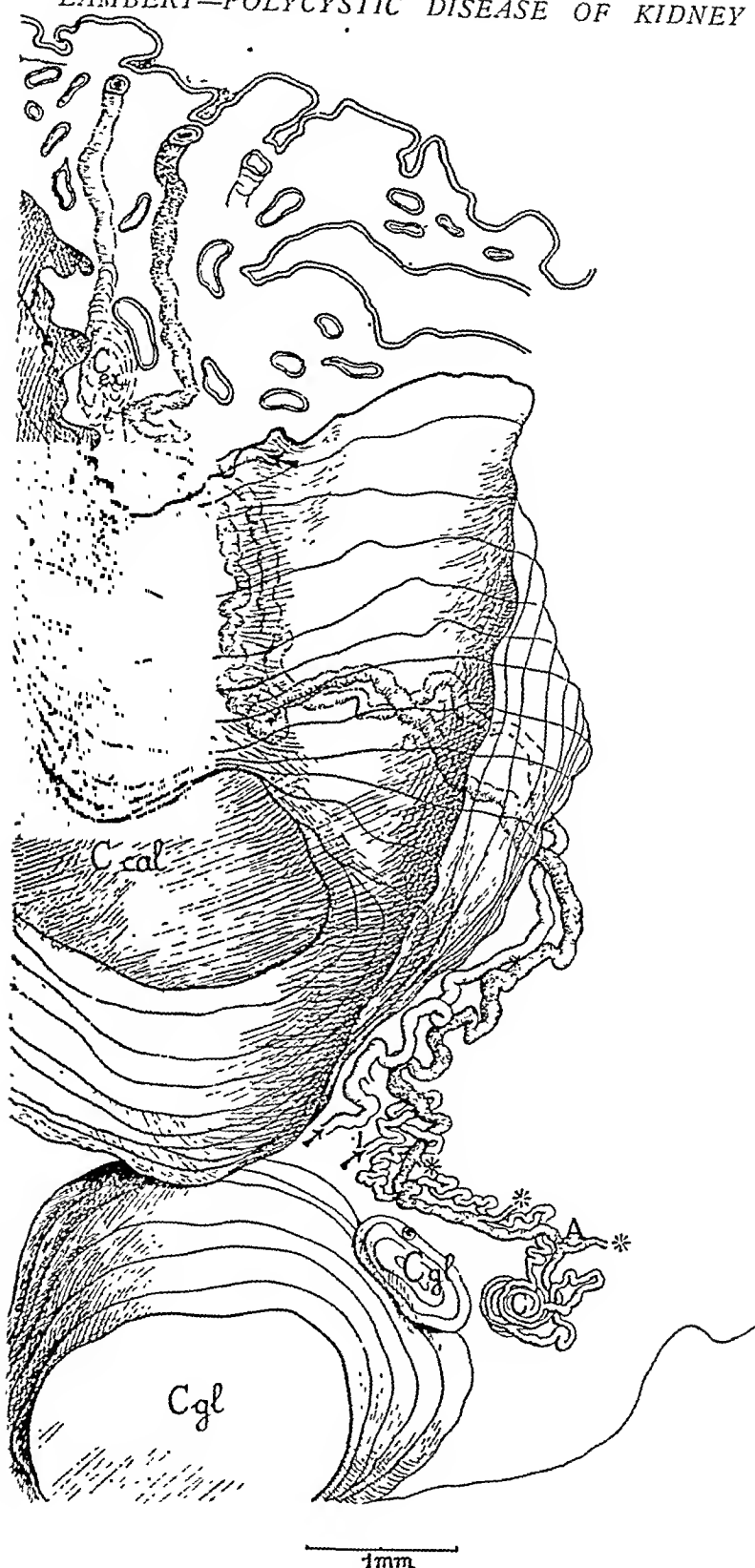
Fig. 8.—Graphic reconstruction of a cystic nephron including the collecting tubule and the duct of Bellini: *C* indicates the cyst; the asterisks, openings of other collecting tubules into the duct of Bellini; *C ex*, a neighboring tripolar excretory cyst; *C gl*, a glomerular cyst; *C cal*, a voluminous cyst of the calix.

Figure 9 shows a so-called multilocular cyst, an arrangement of confluent cavities, often found in the polycystic kidney of the adult. Cysts of this type have been thought to be true adenomatous formations, but in our opinion they do not differ essentially in their mode of origin from other cysts. Serial sections of 2 examples have been examined. In the first a vascular tuft was found, indicating that it was a glomerular polycystic system; the walls, although carefully examined, did not show an issuing tubule. In the second case four openings were found leading to a single Henle's ascending loop. From each of these four cysts there had developed budding daughter cysts so intimately intermingled that an exact drawing of the resulting complexity was impossible.
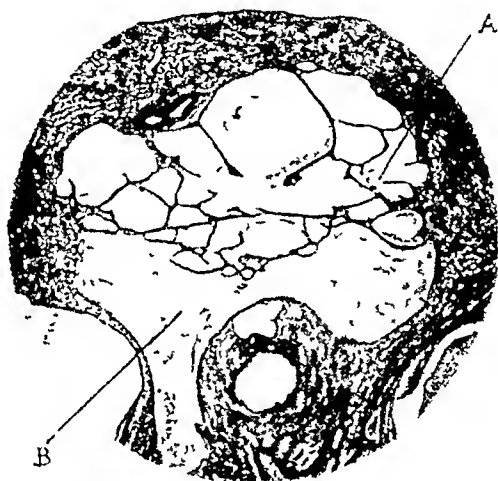
Fig. 9.—Multilocular tubular cyst of the ascending loop of Henle: *A* indicates the proximal portion of the tubule; *B*, the distal portion of Henle's loop

3. Excretory Cysts. Cysts formed from collecting tubules were commonly found in the medulla. Sometimes, like the cysts of the nephrons, they were bipolar, but characteristically they had three or more poles. This is understandable when the dichotomous origin of the collecting tubules is remembered, for a cyst developing at a junction of two tubules will clearly be tripolar, and if several branches are included within a cyst, it will be multipolar. For the most part, these cystic collecting tubules continued in serial sections as undistended ducts of Bellini to open into the calix. Occasionally, however, the dilated tube was pinched off, leaving the cyst isolated from the calix, a feature already described by Ruckert.[14] Rarely the cyst was at the extreme distal portion of the duct of Bellini, where it opened directly into the calix.

CONCLUSIONS

It is evident from this presentation that though renal cysts of the adult and the newborn are similar in essential structure the cystic organs differ in topography. In both the newborn and the adult the glomerular cysts appear as closed systems; in the adult, cysts occurring along the course of the tubules as a rule are connected with the excretory tubules and the renal pelvis.

For this reason we feel that all cysts must develop independently of such factors as a failure of the tubule of the nephron to unite with an excretory channel or an incomplete development of the tubule of the nephron, and also that they are not the result of fibrous tissue closure of collecting tubules.

It must be admitted that the cause and the genesis of polycystic disease therefore remain unexplained, and all that can at present be said is that the previous conceptions of its origin, based on the three factors which have now been eliminated, are erroneous. At least the detailed and exact morphologic description of the cystic kidney which I have given has opened the way for a physiologic assessment of the functional activity of the "cysts" of the adult kidney that is for so long a time able to maintain life, for I have shown that these cystic nephrons open into the excretory system of the pelvis and the ureter. In the following experiments their functional capabilities were examined.

PHYSIOPATHOLOGY OF RENAL POLYCYSTIC DISEASE OF THE ADULT

In the experiments to be described now the following points were examined: (1) the persistence of glomerular activity in the cystic nephrons, (2) the persistence of tubular activity in these nephrons as concerns their ability to reabsorb water, glucose and urea and (3) the persistence of the ability of the proximal convoluted tubule to store colloidal dyes when these substances are introduced directly into the cystic cavity.

GLOMERULAR FUNCTION OF THE CYSTIC NEPHRON

The persistence of glomerular function was studied by injecting large amounts of inulin into the peritoneal cavities of 2 patients a short time before their death. These patients arrived at the hospital with evidence of submeningeal hemorrhage and died fifteen and ninety-five hours after their admission. Forty-one grams of inulin in 5 per cent solution was injected intraperitoneally in both patients immediately on their arrival. With the first patient it was possible to remove by puncture of the two kidneys 4 samples of cystic fluid before the injection of the inulin; with the second, this was impossible. The 4 samples were found to contain nothing that gave a positive reaction for inulin.

The kidneys of these 2 patients were removed immediately after death in order to avoid postmortem diffusion of the components of the urine. Inulin was found in the fluid of most of the renal cysts of both patients in amounts ranging from 10 to 35 mg. per hundred cubic centimeters, while the blood during the eighteen hours following the injection and preceding death showed around 100 mg. of inulin per hundred cubic centimeters. The detailed findings are shown in table 1.

TABLE 1.—*Amounts of Inulin Appearing in the Fluid of Cysts of Both Kidneys, the Calix and the Bladder After Intraperitoneal Injection of This Substance*

		Urea, Gm. per Liter	Inulin, Gm. per Liter
Case 1	Before injection of inulin		
	Cysts of left kidney....................	1.53	0.00
	Cysts of left kidney....................	1.02	0.00
	Cysts of right kidney..................	1.03	0.00
	Cysts of right kidney..................	2.11	0.00
	Eighteen hours after injection		
	Cysts of left kidney....................	1.63	0.00
	Cysts of left kidney.......	0.64	0.00
	Cysts of right kidney..................	2.65	0.10
	Calix	7.48	6.20
	Ninety-six hours after injection		
	Cysts of left kidney....................	1.37	0.25
	Cysts of left kidney....................	0.91	0.17
	Cysts of left kidney....................	2.14	0.34
	Cysts of left kidney....................	1.76	0.16
	Cysts of right kidney..................	2.73	0.20
	Cysts of right kidney..................	2.81	±
	Cysts of right kidney..................	1.76	0.16
	Cysts of right kidney..................	2.34	0.54
	Cysts of right kidney..................	1.66	0.17
	Bladder	14.85	1.74
		Creatinine, Gm. per Liter	Inulin, Gm. per Liter
Case 2	Fifteen hours after injection of inulin		
		0.024	Indeterminate
		0.025	Indeterminate
		0.034	0.103
		0.076	0.075
		0.116	Indeterminate
		0.120	±
		0.175	1.101
		0.200	0.144
		0.288	0.147
		0.380	0.290
		0.576	0.160
		0.960	±
		1.020	0.374

It is easy to understand why greater concentrations of inulin did not occur in the fluid of the cysts since the glomerular filtrate formed during the time that elapsed between the injection and the death was diluted by the large amount of fluid already present in the cyst.

As it is generally admitted that inulin reaches the lumen of the tubule only by way of the glomerulus (Smith [31]), one may assume that its presence in the cystic fluid demonstrates persistence of glomerular function. This conclusion was checked by measuring the hydrostatic pressure of the cystic fluid during a surgical operation on a polycystic kidney. The pressure as measured by means of a water manometer varied from 120 to 180 mm., values lower than the filtration pressure

in the glomerulus, which is about 400 mm., so that there is no incompatibility between our chemical data and this physical finding.

TUBULAR REABSORPTION IN THE CYSTIC NEPHRON

Analysis of fluid removed from the cysts immediately after death makes it possible to demonstrate persistence of tubular function in the cystic nephrons: It has been known for several years that the creatinine which has filtered through the glomerulus is not reabsorbed by the tubules, and more recently it has been shown that endogenous creatinine, conversely to the secretion of exogenous creatinine (Shannon and Ranges [33]), is not secreted by the tubules (Winkler and Parra [34]; Miller and Winkler [35]; Steinitz and Türkand [36]). For this reason it is possible, by finding the urine-plasma ratio of endogenous creatinine, to estimate the amount of reabsorption of water that may occur in the cystic nephron. This was done in 2 cases, and table 2 gives the results in detail. The urine-plasma ratio of endogenous creatinine varies from one cyst to another between 0.7 and 36. This means that reabsorption of water took place in many of the cystic nephrons, probably in those parts of the nephrons proximal to the cysts. The occurrence of a urine-plasma ratio lower than 1 on two occasions in the first case may be explained by the fact that during the last hours of life the concentration of the creatinine of the blood increased and the fact that it may take many hours for an equilibrium to be established between the blood and the fluid of the cysts.

Not only is there evidence that water is reabsorbed in the cystic nephrons but it may also be shown that a part of the urea filtered through the glomerulus returns to the blood as it does in the normal tubule. In the same samples of fluid of the cysts the concentration of urea was determined and the urine-plasma ratio of urea established. This varied from one cyst to another between 0.7 and 7. Since the urine-plasma ratio of urea is lower than that of creatinine this can mean only that when reabsorption of water takes place urea is also reabsorbed. It will be observed that in those cysts where the urine-plasma ratio of creatinine is near 1, the urine-plasma ratio of urea is also about 1, a coincidence which indicates that no urea is reabsorbed when no water is reabsorbed. This conclusion agrees with the generally accepted idea that the reabsorption of urea is a passive back diffusion and not the effect of an active tubular process.

33. Shannon, J. A., and Ranges, H. A.: J. Clin. Investigation **20**:169, 1941.
34. Winkler, A., and Parra, J.: J. Clin. Investigation **16**:869, 1937.
35. Miller, B. F., and Winkler, A. W.: J. Clin. Investigation **17**:31, 1938.
36. Steinitz, K., and Türkand, H.: J. Clin. Investigation **19**:285, 1940.

In figure 10 the reabsorption of the urea of various cysts is compared with the reabsorption of urea observed by Shannon [37] in dogs under different experimental conditions of increasing diuresis. On the vertical axis are plotted the values for

$$\frac{\text{urine-plasma ratio of urea}}{\text{urine-plasma ratio of endogenous creatinine}}$$

and on the base line are plotted the values for the urine-plasma ratio of endogenous creatinine, which varies inversely with the diuresis of the

TABLE 2.—*Concentrations of the Urea and the Creatinine of Samples of the Fluid of the Cysts with the Corresponding Urine-Plasma Ratios*

| | Concentration in Fluid of Cysts | | Urine Plasma Ratio | |
	Creatinine, Gm. per Liter	Urea, Gm. per Liter	Creatinine	Urea
Case 2	0.024	0.53	0.77	0.72
	0.025	0.60	0.80	0.82
	0.034	0.70	1.09	0.95
	0.076	0.76	2.45	1.04
	0.116	0.69	3.74	0.94
	0.120	0.82	3.87	1.12
	0.175	1.14	5.65	1.56
	0.200	1.94	6.45	2.66
	0.288	1.95	9.29	2.67
	0.380	2.21	12.25	3.01
	0.576	2.46	18.50	3.37
	0.960	3.99	31.00	5.46
	1.020	3.06	32.90	4.19
Case 3	0.027	0.85	1.60	1.63
	0.040	0.83	2.35	1.60
	0.041	1.42	2.41	2.73
	0.016	Insufficient	2.72	
	0.058	0.83	3.40	1.60
	0.084	0.83	4.97	1.60
	0.122	1.42	7.18	2.73
	0.153	1.78	9.00	3.42
	0.217	1.48	12.76	2.84
	0.240	1.54	14.10	3.00
	0.245	2.35	14.40	4.52
	0.364	2.85	21.40	5.50
	0.400	3.10	23.50	6.00
	0.555	3.33	32.70	6.40
	0.600	4.15	35.30	8.00
	0.618	3.69	36.40	7.13

dog, according to the data, and is proportional to the reabsorption of the water of the cyst fluid. The results obtained in these two figures are seen to be similar.

Glucose has been found in small amounts in all samples of cyst fluid that have been examined, but the urine-plasma ratio for glucose was lower than 1 in 3 instances and lower than 1.3 in every case in which it was determined. When phlorhizin sufficient to induce glucosuria was injected into a patient twenty-four hours before an operation on the right kidney, the urine-plasma ratio for glucose was found higher than 1.3 in 12 of the 14 samples of cyst fluids removed during the operation (table 3).

37. Shannon, J. A.: Am. J. Physiol. **122**:782, 1938.
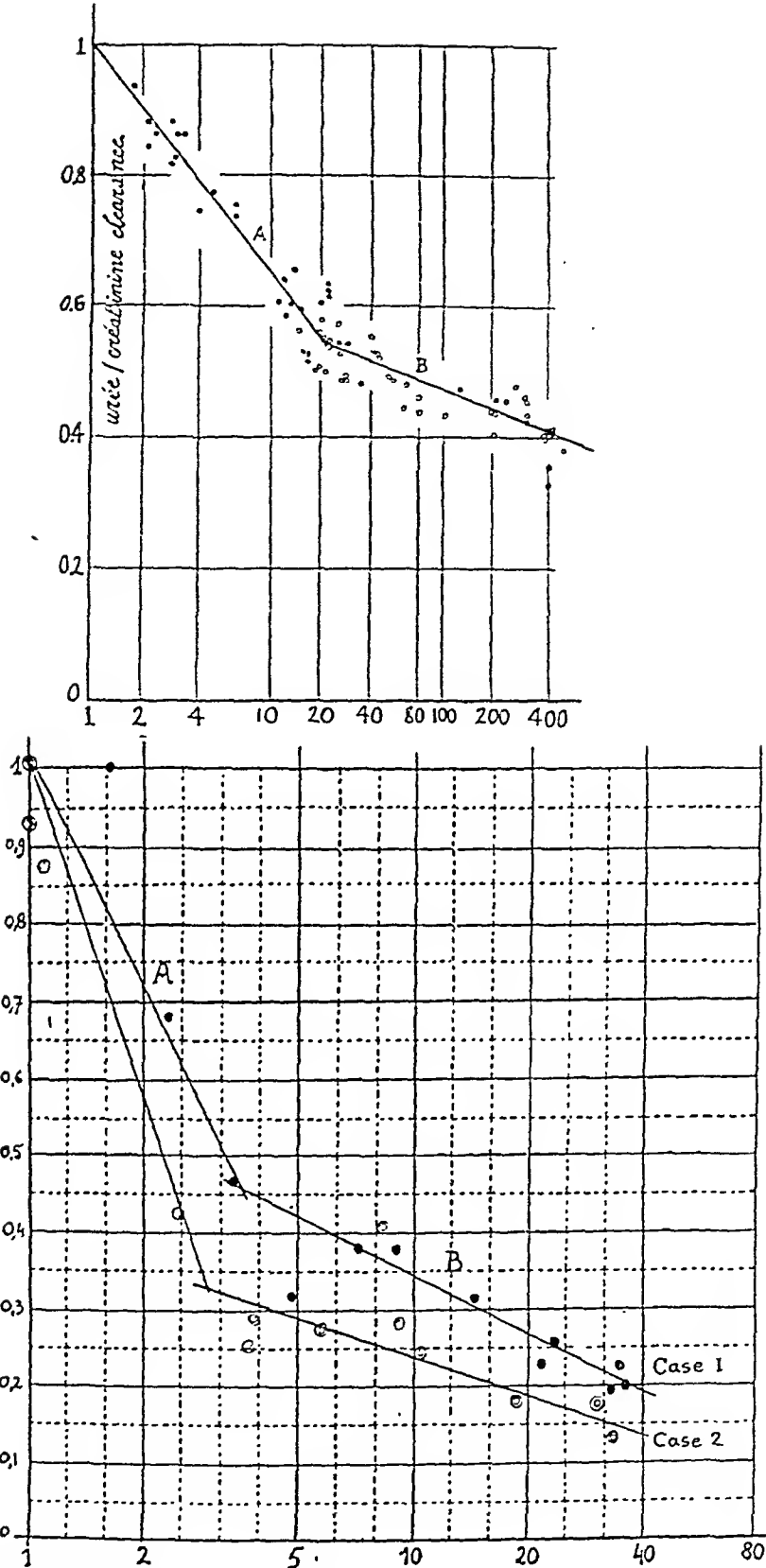
Fig. 10.—Comparison of values for

$$\frac{\text{urine-plasma ratio of urea}}{\text{urine-plasma ratio of creatinine}}$$

at different levels of water reabsorption. The data in the upper figure are taken from Shannon[37] and were obtained from dogs at a time when forced diuresis had been induced and at a time when the output of urine was smaller. The data in the lower figure were obtained from the study of samples of fluid removed from cysts in 2 cases of polycystic kidney. In both figures the urine-plasma ratios of creatinine (horizontal axis) measured the amounts of water reabsorbed by the tubules.

These results indicate persistence of active reabsorption of glucose in those parts of the nephrons proximal to the cysts.

TUBULAR ABILITY TO STORE COLLOIDAL DYES

Finally the ability of the proximal convoluted tubules of the cystic nephrons to store colloidal dyes such as trypan blue and india ink has been examined. In studying this phenomenon in the kidney of Salamandra maculata some years ago with Gerard and Cordier [38] I observed that trypan blue is stored in the proximal part of the convoluted tubule

TABLE 3.—*Concentrations of the Glucose and the Creatinine of Samples of the Fluid of Cysts Taken from the Right Kidney During Phlorhizin-Induced Glycosuria and from the Left Kidney Six Months Later (Case 3)*

Creatinine, Gm. per Liter	Urine-Plasma Ratio of Creatinine	Glucose, Gm. per Liter	Urine-Plasma Ratio of Glucose
	Right Kidney: After Phlorhizin *		
0.040	2.35	1.17	1.42
0.041	2.43	1.56	1.90
0.046	2.72	1.86	2.27
0.057	3.35	Insufficient	Insufficient
0.084	4.97	0.97	1.18
0.122	7.18	0.76	0.93
0.153	9.00	2.53	3.03
0.217	12.76	Insufficient	Insufficient
0.240	14.10	0.92	1.12
0.245	14.40	1.69	2.06
0.364	21.40	1.14	1.40
0.400	23.50	1.55	1.88
0.556	32.70	1.66	2.02
0.600	35.30	1.82	2.22
0.618	36.40	1.79	2.18
	Left Kidney: Without Phlorhizin		
0.021	1.23	0.62	0.750
0.022	1.30	0.21	0.250
0.025	1.47	0.28	0.340
0.028	1.64	1.10	1.340
0.029	1.70	1.09	1.330
0.030	1.76	1.01	1.230
0.039	2.30	0.13	0.150

* The samples from the right kidney were collected during an operation performed twenty-four hours after the injection of phlorhizin. The samples from the left kidney were removed immediately after death six months later.

next to the glomerulus, while india ink is stored more distally in the same convoluted tubule. This difference was shown to be due to the fact that india ink is composed of larger-sized particles than is trypan blue.

A mixture of these two substances was injected into a cyst by renal puncture shortly before the death of one of the aforementioned patients. By a fortunate circumstance the dye in the lumen of a nephron opening into the injected cyst was found in serial sections.[29a] Although both dyes were found in the lumen of the glomerular part of this nephron proximal to the cyst, only trypan blue was stored in the cells at this level of the tubule, while in the more distal part of the tubule cellular

38. Gerard, P., and Cordier, R.: Arch. de biol. **43**:367, 1932; Biol. Rev. **9**:110, 1934. Lambert, P. P.: Arch. de biol. **47**:125, 1936.

storage was limited to india ink. This finding reproduces exactly what was observed in the kidneys of the salamander when the dyes were introduced into the lumens of nephrons which open into the peritoneal cavity by means of nephrostomes. So far as we know this is the only instance on record in which tubular cells of the human kidneys stored a substance with as large particles as india ink, for normally india ink does not reach the lumen of the tubule because it cannot pass through the glomerular membrane.

CONCLUSIONS

From these experiments it appears that the cystic nephrons of the adult retain a considerable part of their functional ability, and there is no reason to doubt that they take part in the formation of urine.

This cannot be true of the cystic kidney of the newborn. Although the, function of the cystic nephrons of infantile kidneys has not been examined by experiments similar to those applied to the cystic nephrons of adult kidneys, the nephrons have been carefully examined by morphologic methods and in the observations so far presented appear as closed systems, isolated from the pelvis of the kidney. As a result, the infant dies promptly, often before birth, while the adult survives, often to late middle age. Our observations answer satisfactorily a question that frequently arises when one is considering the last stages of polycystic disease in the adult, namely: Where is the functioning renal parenchyma, and where is the urine coming from? As shown in the foregoing pages, the cystic open nephrons are able to provide for the formation of urine to a certain extent and this function they doubtless maintain in the last period of life when the neighboring non-cystic parenchyma has been destroyed either by the enlarging cysts or by the development of sclerotic tissue.

SUMMARY

Graphic reconstructions of polycystic kidneys of the newborn have shown that the cysts are closed cavities consisting of distended nephrons which are isolated from the pelvis, a conclusion which agrees with the opinion of previous investigators.

The same method of examination applied to the adult type of the polycystic disease showed glomerular cysts, which appeared as closed cavities, and tubular cysts, which had developed in the course of the nephrons.

When the cystic nephrons were examined in serial sections and in their reconstructed continuity it was found that most of them were completely developed and connected in the usual way with the collecting tubules and that these opened freely into the pelvis.

The tubular cyst appeared in the course of the proximal convoluted tubules, in Henle's loops, in the distal convoluted tubules and also in

the medulla along the course of the collecting tubules. From the morphologic point of view it therefore seemed that they might well have retained a part of their renal function. This was investigated by physiologic methods.

Inulin appeared in the cysts after intraperitoneal injection, an indication of glomerular filtration. The fluid from the cystic tubules was found to have the essential characteristics of the urine. Its creatinine level was typically many times higher than that observed in the blood, an indication that water was being absorbed by the cystic nephrons. The same observation was found to be true of urea. Moreover, a back diffusion of urea in the cystic nephrons similar to that which occurs in the normal nephron could be demonstrated. Although the fluid of the cysts contained small amounts of glucose, the concentration of this component increased after the injection of phlorhizin.

And lastly, colloidal substances injected directly into the cyst were stored in the cells of the convoluted tubule showing that this peculiar function of the proximal convoluted tubule had persisted in the cystic nephron.

BOVINE LEPTOSPIROSIS

Pathologic Observations on Experimentally Infected Calves

H. UNGAR, M.D.

AND

H. BERNKOPF, M.D.

JERUSALEM, PALESTINE

RECENTLY it was shown that a disease of cattle of Palestine. characterized by fever and. when severe, by jaundice, was caused by a Leptospira which differs serologically and in its pathogenicity from Leptospira icterohaemorrhagica and Leptospira canicola.[1] Cases of severe leptospirosis of man caused by the same strain were also reported.[2] The disease was successfully transmitted to calves through several passages, and the clinical picture has been reported, together with the changes in the blood and the urine of infected animals. The pathologic-anatomic findings in this series of animals ánd in additional animals of later experiments are the subject of the present study.

REVIEW OF THE LITERATURE

Knowledge of leptospirosis of cattle is of relatively recent date, and literature on the pathologic aspects of this disease is scarce.

Jungherr[3] described observations made on 3 young cows dying of a "mysterious" disease which at autopsy revealed lesions similar to those seen by the same author in cases of canine leptospirosis. The diagnosis was supported by the histologic demonstration of "leptospira-like bodies" in 3 cases, in the kidneys, in a liver and in a mesenteric lymph node. Marsh[4] succeeded in demonstrating leptospiras in histologic sections of liver and kidney taken from a calf which died during an epidemic of icterohemoglobinuria. In his report the histologic changes were only briefly mentioned. More recently. Mathews[5]

1. Bernkopf, H.: Harefuah **30**:110, 1946. Bernkopf, H.; Olitzky, L., and Stuczinski, L. A.: J. Infect. Dis. **80**:53, 1947.

2. Padersky, B.: Harefuah **30**:117, 1946. Btesh, S.: ibid. **30**:121, 1946. Ephrati, P.: ibid. **30**:125, 1946.

3. Jungherr, E.: J. Am. Vet. M. A. **105**:661, 1944.

4. Marsh, H.: J. Am. Vet. M. A. **107**:119, 1945.

5. Mathews, F. P.: Am. J. Vet. Research **7**:78, 1946.

reported on the necropsies of cattle dying during a field epidemic. The author found leptospira-like organisms in sections of liver and kidneys and recognized a certain similarity between the histologic changes in cattle and those in dogs with leptospirosis. He hesitated, however, completely to accept the etiologic role of leptospiras, because of excessive variations in the clinical picture, which in its acute-fatal, moderate and chronic forms conformed neither to the intensity of the damage of tissues nor to the number of leptospiras in the tissues. The author attempted to transmit the disease by subcutaneous injections of blood and of emulsions of kidney and liver from diseased animals. A febrile disease was thus produced in 7 calves, but the animals were not killed for pathologic examination. Transmission experiments in which sheep and goats were used gave negative results, while two guinea pigs became ill, but no characteristic lesions of tissues developed, and no spirochetes could be demonstrated.

An earlier and extensive study of the anatomic findings in bovine leptospirosis was published by Avrorow,[6] in 1941, but unfortunately only an abstract of this author's paper was available to us. During an epidemic occurring in the Caucasus he examined more than 40 field cases of leptospiral jaundice, as well as 4 experimentally infected calves. The lesions in the latter were reported to conform with those in the spontaneous cases, and spirochetes were demonstrated in histologic sections of several organs.

MATERIAL STUDIED

Twelve calves were available for examination in the present study. At the time of infection the animals were about 10 to 14 days of age. One of them (calf 44) had been experimentally infected with blood taken from a cow at the beginning of a "spontaneous" attack of jaundice, in the preicteric stage, and 8 calves were subsequently infected in consecutive passages. Three further animals were inoculated with a pure culture of the strain of leptospiras which was isolated from the blood of a human victim of the disease and which behaved serologically and in its pathogenicity exactly as did the strain isolated from infected calves of the first series of experiments.[7]

At necropsy, pieces of liver, spleen and kidney were fixed for histologic examination in Zenker's fluid as well as in 10 per cent formaldehyde solution. Sections were stained with Ehrlich's hematoxylin-eosin, iron hematoxylin-Van Gieson, Heidenhain's azan, Foot's silver impregnation or sudan III; blocks of liver and kidney were impregnated with silver according to Levaditi's method.

Bacteriologic cultures were made from the blood and organs, and leptospiras were isolated from the blood of several animals. Paratyphoid-B bacteria were isolated from the organs of 4 animals and were found to belong to four serologically different groups. In no instance had this infection, which is rather common in Palestinian cattle, been suspected on clinical grounds. The presence of blood parasites was excluded by the examination of smears from blood and hemopoietic organs.

6. Avrorow, A. A.: Ztschr. f. Veterinärk. **53**:32, 1941.

7. Bernkopf, H.; Stuczinski, L. A.; Gottlieb, T., and Halevy-Katz, C.: To be published.

FINDINGS

The infections of our animals did not run a uniform course.   Three separate clinical pictures could be differentiated, and our material was accordingly grouped.   The first group included 6 animals (3 from the blood passage series and 3 infected with a pure culture of the strain of leptospiras studied) in which fatal jaundice developed.   To the second group belong 2 animals which died with no jaundice developing.   Four animals in which a mild disease developed and which recovered constitute a third group.   Additional details are given in each section.

GROUP I.—These animals died of the fulminating form of the disease, which was accompanied by jaundice (calves 44, 45, 55, 95, 96 and 106).

Summary of Clinical Observations.—Calf 44 was inoculated with blood taken from a diseased cow in the preicteric stage.   The calf had jaundice ten days after inoculation and died on the eleventh day.   Blood from this animal was transmitted to calves 45 and 52, and blood from the latter, to calf 55.   Calves 95, 96 and 106 were inoculated with a pure culture of bovine leptospiras isolated from a human subject.   The disease ran the same course in all the animals of this group.   Blood urea was high in the terminal stages in all cases.   In calves 55, 95 and 96 the jaundice was accompanied by a sudden fall in the erythrocyte count.   The animals died between the seventh and the tenth day after inoculation.

Some Details of the Necropsies.—Serous and mucous membranes and adipose tissues were jaundiced in varying degrees.   The liver was of normal size and consistency, and its color was normal or slightly yellowish.   The cut surface of the liver revealed no gross changes.   The spleen was slightly softer than usual.   The kidneys were without macroscopic changes except for an icteric tinge and moderate hyperemia of the cortex.   The urinary bladder contained dark and reddish brown urine.   There were scattered hemorrhagic areas in the lungs.   Bacteriologic culture of the organs revealed a paratyphoid-B infection in calves 44 and 55.   Cultures of the blood used for further animal passages showed no bacterial growth.

Microscopic Observations.—(*a*) Liver: There was marked hyperemia of the sinusoids and of the portal branches.   The structure was well preserved except in scattered areas where the cells were detached from one another; in 1 animal (45) this lesion had spread over extensive areas (fig. 1*A*).   Small groups of liver cells were slightly enlarged and showed discrete vacuolation of the cytoplasm.   Fatty change could not be demonstrated with sudan III.   Only in 2 calves (95 and 55) was necrosis of liver cells observed.   In 1 animal (95) discrete scattered foci of necrosis were present, and these were infiltrated by polymorphonuclear leukocytes.   They were seen especially in the vicinity of central veins (fig. 1*B*).   In calf 55, from which paratyphoid bacilli had been isolated, fairly large necrotic nodules were seen.   In contrast to the necrotic foci in calf 95, the nodules in calf 55 included cells with large vesicular nuclei and with only a few granulocytes in addition to nuclear debris.   When the necrotic nodules were adjacent to larger branches of the hepatic vein, there was evidence of phlebitis and mural thrombosis.

Kupffer cells were generally small, with dark nuclei.   Some irregularly distributed Kupffer cells, however, were enlarged and rounded, and exhibited vacuoles and inclusions which resembled erythrocytes in their size and in their manner

of staining with eosin. The prussian blue reaction for hemosiderin was positive in the majority of Kupffer cells. Sparsely distributed, discrete sudanophil granules were demonstrated in them with sudan III. Macrophages containing hemosiderin were seen in the periportal tissue in several animals. In its smaller ramifications the periportal tissue displayed infiltrating cells, mainly accumulated around bile ducts. The latter frequently contained bile thrombi (fig. 1 D) which in some instances included desquamated epithelial cells. The accumulations of cells were composed chiefly of lymphocytes, a few plasma cells and histiocytes, and in a
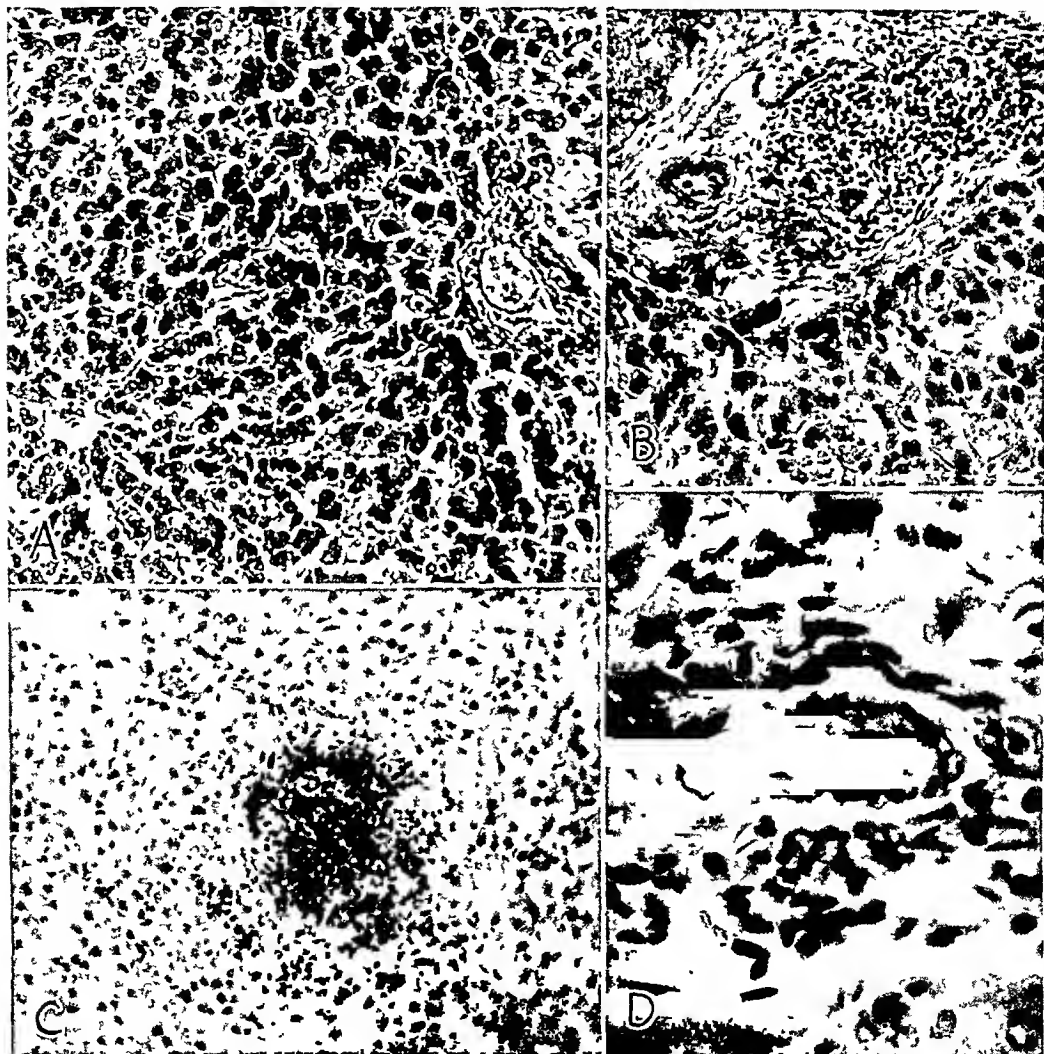
Fig. 1.—*A*, dissociation of liver cords of calf three days after onset of severe jaundice. × 140.

B, cholangitis occurring in the liver shown in A. × 125.

C, necrotic focus infiltrated with polymorphonuclear leukocytes in the liver of a calf dying during an attack of severe jaundice. × 170.

D, cholangitis and bile thrombus in the liver of a jaundiced calf. × 560.

few places some polymorphonuclear leukocytes were also present (fig. 1 *C*). The closeness of the infiltrations to the bile ducts and the observed changes within the latter justify the diagnosis of cholangitis.

The sections treated by Levaditi's method revealed leptospiras in the livers of the jaundiced animals in sparse numbers only. Some organisms were found within liver cells; others were seen among detached cells or lying near the walls of sinusoids (fig. 2 B). In the latter instances the characteristic hook of the single, well preserved organism made it possible to distinguish it from the reticular fibers which sometimes became impregnated with silver. In 1 calf (106) clusters of leptospiras were demonstrated in the blood of the larger ramifications of the portal vein (fig. 2 A).

(b) Spleen: The malpighian bodies were small, without germinating centers. The sinusoids were narrow, and their endothelium appeared normal. The intersinusoidal tissue contained abundant erythrocytes and moderate and approximately equal numbers of polymorphonuclear leukocytes and reticular cells. The prussian blue reaction was positive in small, irregularly distributed groups of cells. In calf 55, which was found to harbor a latent paratyphoid infection, the spleen contained nodular necrotic foci containing nuclear debris.

(c) Kidneys: The glomeruli were moderately hyperemic, and single erythrocytes were occasionally present within Bowman's capsule. In many areas the tubules were distended, and albuminous and leukocytic casts occurred, especially in the straight portions. The epithelium exhibited a fair number of necrotic cells in irregular distribution.

In 2 animals (45 and 95) the epithelium had undergone remarkable changes which require more detailed description. In extended portions of convoluted tubules the cells were enlarged, the nuclei being in a basal position, the cytoplasm showed marked vacuolation and contained pigment, which took a reddish tinge like that of hemoglobin when stained with eosin (fig. 2 C). In some instances several small coalescing vacuoles contained spherical elements which resembled erythrocytes in size and outline; they stained only faintly in the center but were defined by a bright reddish rim. The prussian blue reaction was strongly positive in many of these vacuolated cells as well as in several casts containing desquamated epithelial cells. The stain for hemoglobin recommended by Dunn and Thompson [8] was tried, but with negative results.

In all animals focal intertubular accumulations of cells were present (plasma cells, large histiocytes, lymphocytes and a few polymorphonuclear leukocytes). The infiltrations were found mainly between convoluted tubules, sometimes approaching the external border of Bowman's capsule. In rare instances, when the infiltrations had broken into tubules the latter contained leukocytic casts.

Silver impregnation revealed moderate numbers of leptospiras in the epithelium of tubules and in the tubular lumens.

GROUP II.—These animals died after a moderately prolonged course of the disease, which was unaccompanied by jaundice (calves 65 and 76).

Summary of Clinical Observations.—Calf 65 was infected with the blood of the jaundiced calf 55, and calf 76, with blood taken from calf 69 (see group III) during the febrile period. Both animals died without development of jaundice twenty-one and sixteen days, respectively, after inoculation. They had high temperatures, leukocytosis, high blood urea and, transiently, traces of bilirubin in the serum. Leptospiras were cultured from the blood of both animals.

Some Details of the Necropsies.—The tissues were not jaundiced. The liver was dark brown and the gallbladder moderately filled. The kidneys were markedly congested, and their cut surfaces were reddish brown. The spleen was without

8. Dunn, R. C., and Thompson, E. C.: Arch. Path. **39**:49, 1945.

gross abnormalities.   Subserous petechiae were seen over the entire surface of the stomach, and small hemorrhages were observed in the gastric mucosa.

Bacteriologic examination of the organs revealed paratyphoid-B infections due to serologically distinct strains in both animals.
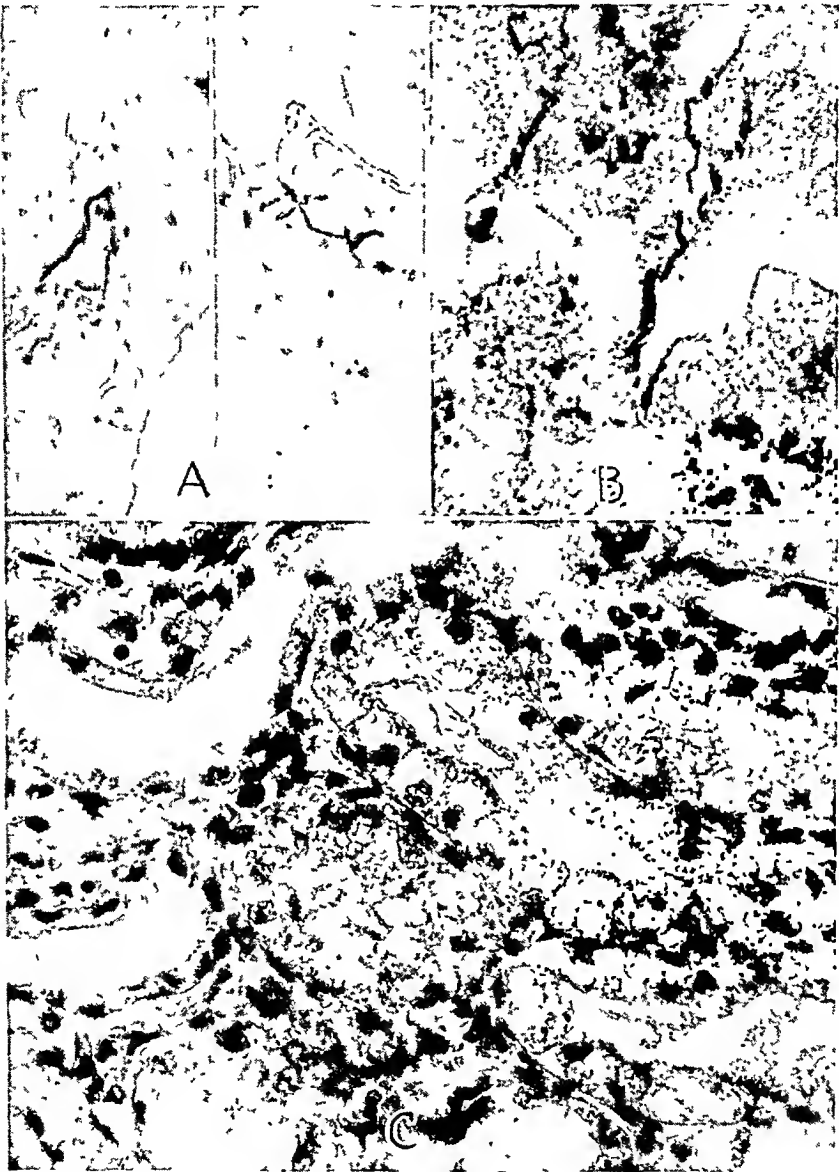
Fig. 2.—*A*, leptospiras lying within the lumen of a portal vein of the liver of a jaundiced calf.   × 1,400.

B, organisms lying between liver cells.   × 1,400.

C, kidney of a calf with severe jaundice accompanied by hemoglobinuria.   The tubular epithelium showed a diffuse reddish tinge like hemoglobin and vacuoles containing droplets staining with eosin as do erythrocytes.   × 350.

Microscopic Observations.—(*a*) Liver: The acinous structure and the hepatic cords were left intact except for moderate centroacinic dissociation in calf 76.

There were scattered necrotic foci as previously described in the case of calf 55, which were considered to be so-called "paratyphoid nodules." Moderate hypertrophy of the Kupffer cells was noted. The periportal tissue contained scattered histiocytes which gave a positive prussian blue reaction. Seveal small bile ducts contained bile thrombi, and adjacent to them appeared accumulations of plasma cells, lymphocytes and scattered polymorphonuclear leukocytes such as were observed in jaundiced animals. Levaditi's stain showed single leptospiras within intact liver cells.

(*b*) Spleen: The malpighian corpuscles seemed normal. The endothelium of the sinusoids was flattened, and the lumens were not dilated. Reticular tissue contained numerous reticulum cells, small dark round cells and plasma cells in moderate numbers. The prussian blue reaction revealed abundant macrophages containing hemosiderin granules in calf 65.

In both animals irregularly distributed nodular necrotic foci of the type described as paratyphoid nodules were seen.

(*c*) Kidneys: The glomeruli were without abnormalities. The tubules were normally wide, while in convoluted portions the epithelial cells were swollen, displaying granulated cytoplasm and a moderate loss of nuclei. Now and then the cortex was found to include intertubular accumulations of lymphocytes and a considerable number of plasma cells. In some areas there was evidence that the infiltrating cells had penetrated into tubules which contained leukocytic casts. Levaditi's stain disclosed single leptospiras in' the lumens of tubules close to the epithelial border.

GROUP III.—These animals achieved clinical recovery after a mild attack (calves 60, 61, 64 and 69).

Summary of Clinical Findings.—Calves 60, 61 and 64 were infected with blood of the jaundiced calf 55. They reacted with fever, leukocytosis and a slight rise of blood urea. All 3 animals recovered. They were killed outside the laboratory one week after the fever had subsided, i. e., twenty-one days after inoculation.

The fourth animal of this group (69) was infected with blood of calf 65 (see group II). It reacted, as did the other 3 animals, with fever and leukocytosis for several days, and there were transient traces of bilirubin in the serum. The blood urea rose to 129 mg. per hundred cubic centimeters on the twelfth day after inoculation. This animal was killed for examination while in a state of perfect clinical health fifty-nine days after inoculation.

Some Details of the Necropsies.—All the animals were free of macroscopic changes except calf 69, which exhibited severe renal damage. The kidneys of this animal were considerably enlarged, weighing 550 Gm. each. They were of doughy consistency, and their cut surfaces were moist and pale reddish gray. No abnormalities were found in other organs. No bacterial infection was observed in the blood and organs of these animals.

Microscopic Observations.—(*a*) Liver: There were identical findings in all the 4 animals of this group. The acini were well formed, but the trabeculae frequently were irregular in outline because of cellular hyperplasia (fig. 3 *A*). Multinuclear cells were rare, and mitotic figures were not found.

The periportal tissue was sparsely developed and poor in cells. Kupffer cells were flattened and had dark nuclei. Hemosiderin stains and fat stains ·gave negative results. No leptospiras were found.

(*b*) Spleen: The malpighian corpuscles showed no abnormalities. There was pericorpuscular hyperemia, accompanied by accumulations of polymorphonuclear

leukocytes. The sinusoids were widely open and lined with small dark endothelial cells. In 1 animal the sinusoids were small and seemed more numerous than usual, suggesting the presence of so-called sinus hyperplasia. The reticular tissue was sparsely developed and contained scattered plasma cells. The prussian blue reaction was negative.

Fig. 3.—*A*, liver of a calf which was not jaundiced, from group III. Note the hypertrophy of the liver cords, which may be attributed to hyperplasia of liver cells. Foot's silver impregnation; × 450.

B, kidney of a calf 59 days after inoculation. This animal recovered completely after slight illness. Note severe interstitial nephritis breaking into the tubules. × 160.

(c) Kidneys · The kidneys of calves 60, 61 and 64 revealed no changes except for occasional loss of nuclei of the epithelium of the convoluted tubules. Leptospiras could not be demonstrated.

In the kidney of calf 69 microscopic lesions were observed as follows:

The glomeruli were without abnormalities. In all parts of the kidney there was an abundant interstitial cellular infiltrate, predominantly composed of plasma cells and lymphocytes. The central portions of some foci of infiltration contained accumulations of polymorphonuclear leukocytes. In several areas, mainly in the pyramidal portions, small abscesses were present The tubules adjacent to cellular infiltrations frequently contained leukocytic casts (fig. 3 B). Silver impregnation revealed abundant leptospiras in the epithelium and in the lumens of convoluted tubules in areas which were free of inflammatory exudate. Many fragmented
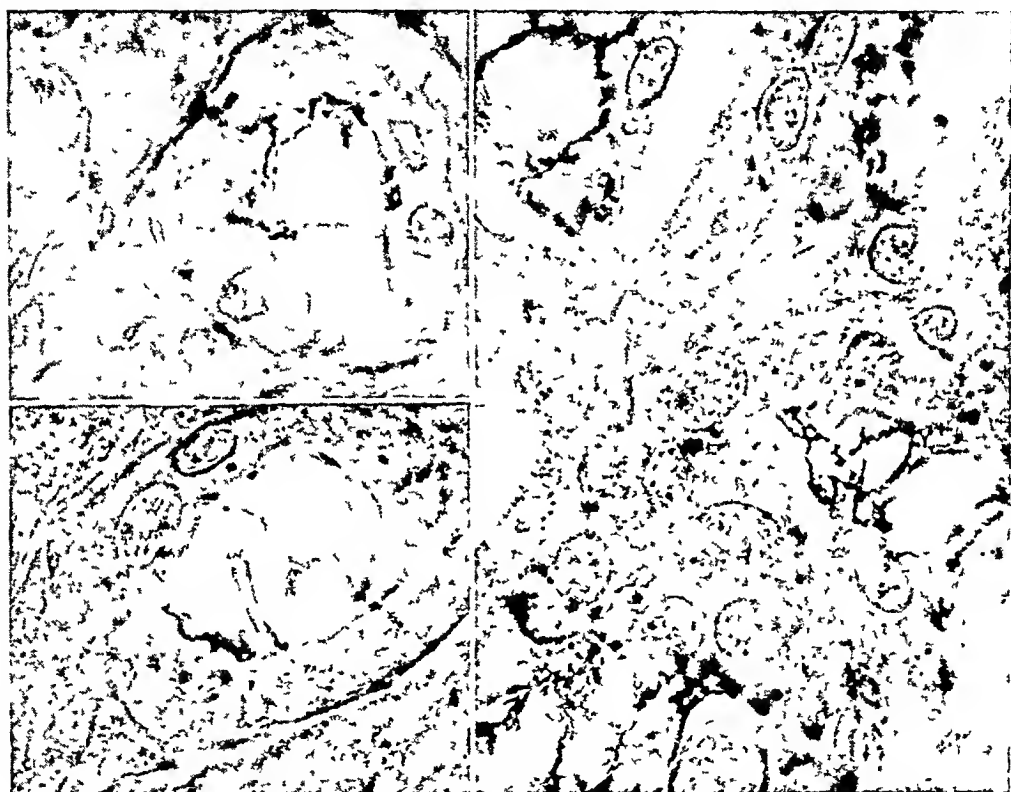
Fig. 4.—Leptospiras in renal tubules of the kidney shown in figure 3 B. × 1,400.

leptospiras were present, together with typical complete organisms. In some places the entire lumen of a tubule was lined with a thick network of leptospiras (fig. 4).

COMMENT

Nearly all previous reports on the pathologic aspects of bovine leptospirosis have dealt with observations in field cases. It seems, therefore, worth while to compare these observations with ours, made on experimentally infected animals.

Changes in the Liver.—In the livers of 7 of 8 calves which succumbed to leptospirosis in its fulminant or in its protracted form, dissociation

of hepatic cells was noted, while necrotic foci were found only three times. This experience is not shared by other authors. Dissociation was also recorded by Jungherr,[3] but the low incidence of regressive changes of liver cells in our material does not agree with the experience of others,[9] who reported the frequent occurrence of necrotic foci. Moreover, such foci were seen by us, with only a single exception, in animals harboring paratyphoid bacilli and were probably caused by this concurrent infection. This view is supported by the histologic characteristics of the necrotic foci, which are identical with the well known paratyphoid nodules,[10] by their simultaneous presence in the spleen and by their occurring whether the animals were jaundiced or not.

There remains only a single case in which necrotic foci were found in the liver of an animal which died at the height of a rapidly fatal attack of jaundice and was found free of concurrent infection. These foci were different from the paratyphoid nodules by their centroacinic location, their small size and the presence of polymorphonuclear leukocytes.

In our experience the periportal tissues seemed to be more constantly injured than the hepatic epithelium. In these tissues, infiltrations of small round cells were found, predominantly adjacent to the bile ducts, which frequently contained bile plugs and desquamated epithelium, and the diagnosis of cholangitis thus seemed justified. Periportal infiltrations were also mentioned by Avrorow. A possible explanation of this observation may be found in the occurrence of abundant leptospiras in the portal vein, as seen in an animal of our series. It is conceivable that the low pressure of the portal circulation allows the blood-borne leptospiras to be in prolonged and intimate contact with the periportal tissues.

Parenchymal retention of bile was found in the liver of only a single animal, which had necrotic foci in the liver in the absence of paratyphoid infection. The retention of bile seemed therefore to play only a minor role in the production of jaundice.

The hemosiderin present in the reticuloendothelial cells and in the liver cells of jaundiced animals points to the important role played by the destruction of red blood cells in the origin of jaundice. Further evidence of this destruction lies in the fact that in 3 of 6 jaundiced animals the erythrocytes of the peripheral blood decreased in number suddenly at the time of the appearance of the jaundice.

Changes in the Spleen.—The lesions of the spleen were negligible and not specific in nature. In the grave icteric form as well as in the protracted nonicteric form of the disease congestion and hemosiderosis

9. Mathews.[5] Avrorow.[6]

10. Joest, E.: Leber und Gallenwege, in Frei, W., and others: Joest's Handbuch der speziellen pathologischen Anatomie der Haustiere, ed. 2, Berlin, Verlagsbuchhandlung von Richard Schoetz, 1936, vol. 2, pt. 1.

were found. There were no abnormalities in animals which recovered following a mild illness. In no instance could leptospiras be demonstrated.

Our own experience corresponds to that of other authors, except Mathews, who found atrophy of the lymphoid tissue and persistent hemosiderosis in cases with infection lasting two to three weeks. Our, on the whole, negative findings agree also with reports on canine leptospirosis and on human cases of Weil's disease.

Changes in the Kidneys.—In the acute icteric form a moderate degree of degeneration of the tubular epithelium was present. In several jaundiced animals a peculiar vacuolar change of the epithelium was seen, accompanied by different kinds of pigmentation, which had also been observed and described by Mathews. As in his case, we observed pigmentation giving a positive iron reaction in certain portions of the tubules. In others, the epithelial cells contained pigmented iron-negative vacuoles which stained with eosin precisely as did erythrocytes but failed to stain specifically with the Dunn-Thompson method recommended for the demonstration of hemoglobin pigment.

In addition to the regressive lesions, interstitial infiltrations were found in the kidney as early as seven days and as late as fifty-nine days after inoculation. The infiltrations appeared in both jaundiced and non-jaundiced animals. They were present only in kidneys containing leptospiras. The organisms were not found within the areas of infiltration but only within renal tubules with well preserved epithelium.

Interstitial nephritis was observed in leptospirosis of cattle by Mathews. He observed this lesion only when the clinical manifestations of the illness had lasted longer than two to three weeks. The question arose whether the interstitial nephritis which appeared as early as the first few days of illness was really due to the presence of leptospiras or to injury not related to these organisms.

In particular it was necessary to exclude the possibility of interstitial nephritis, which is endemic among young calves in some countries. This condition is known as "white-spotted kidney."[11] We felt that the presence of this disease could be excluded for various reasons: The kidneys of our experimental calves never showed the "whitish patchy discoloration" of the surface described by Theobald Smith. This author considered the disease to be probably due to Bacillus coli infection of newborn calves, but that organism was never found at autopsy in our cases. Furthermore, the endemic form of interstitial nephritis is said to heal spontaneously with residual scars during the second month of life, whereas in our cases infiltrations were present without evidence of fibrosis up to the end of the third month.

---

11. Smith, T.: J. Exper. Med. **41**:413, 1925. Nieberle, K.: Interstitielle Nephritis, in Nieberle, K., and Cohrs, P.: Lehrbuch der speziellen pathologischen Anatomie der Haustiere, Jena, Gustav Fischer, 1931.

The lesions observed in calves when disease is acute are essentially identical with those seen in human patients infected with leptospiras from bovine leptospirosis [12] and in Weil's disease.[13] In regard to the human infection, the absence of degenerative lesions of the liver and the small round cell infiltration of the periportal tissue are stressed by most authors.

Interstitial nephritis as seen in our animals has long been known to occur in patients with Weil's disease, even in nonjaundiced ones.[14] The major importance of renal as compared with hepatic lesions has again been emphasized recently with regard to the experimental infection of guinea pigs with Leptospira hemorrhagica.[15]

In the bovine as in the human disease there is no apparent correlation between the intensity of the structural lesions and the clinical manifestations of the disease; the lesions found in our material were almost identical in the jaundiced and in the nonjaundiced calves except that varying degrees of hemosiderosis were observed in the jaundiced group. The diseases in man and cattle also have in common the fact that leptospiras occur in the liver and in the kidneys independently of cellular infiltrations. Further, it is found in both the human and the bovine material that the leptospiras persist in the kidneys for several weeks in cases distinguished by a mild clinical course.

SUMMARY

Young calves experimentally infected with a bovine strain of leptospiras were examined at necropsy. A description is given of the structural changes observed in the liver, the spleen and the kidneys in three different clinical forms of the disease (an acute form accompanied by jaundice, a subchronic form without jaundice and a form with a mild course leading to clinical recovery).

Leptospiras were demonstrated by the Levaditi method in the liver and the kidneys of every animal in the jaundiced and the subchronic, nonjaundiced group. They were also present in a kidney of 1 calf two months after inoculation. This animal suffered only a slight attack and appeared to recover completely.

The histologic lesions observed were essentially similar to those reported in human Weil's disease.

12. Caspar, J., and Reif, L.: Harefuah **30**:113, 1946.

13. Jeghers, H. J.; Houghton, J. D., and Foley, J. A.: Arch. Path. **20**:447, 1935. Ashe, W. F.; Pratt-Thomas, H. R., and Kumpe, C. W.: Medicine **20**:145, 1941.

14. Stiles, W.; Goldstein, J. D., and McCann, W. S.: J. A. M. A. **131**:1271. 1946.

15. Wylie, J. A. H.: J. Path. & Bact. **58**:351, 1946.

MEDULLARY HYPERPLASIA OF THE ADRENAL GLAND IN AGED WISTAR ALBINO AND GRAY NORWAY RATS

ELEANOR H. YEAKEL, Ph.D.
PHILADELPHIA

THIS paper presents a description of medullary hyperplasia of the adrenal gland observed in old animals that were studied in a cooperative investigation of changes occurring with age in the tissues and organs of rats. Findings based on the weights of the adrenal glands, the relative volumes of cortex and medulla and the histologic alterations of the cortex will be reported separately.[1]

MATERIALS AND METHODS

Rats of the Wistar albino and the gray Norway strain, born and raised in the Wistar colony, were used. In this study the old animals ranged from 700 to over 1,000 days of age, and included 16 female and 15 male albino and 3 male and 3 female gray Norway rats.

The adrenal glands were dissected free of connective tissue, and, except in 1 instance, the two glands were weighed together. One of the adrenal glands from each animal was fixed in Bouin's fluid, sectioned serially and stained with hematoxylin and eosin. The other gland was fixed in Zenker's fluid, and sample sections were stained either with hematoxylin and eosin or with Masson's or Mallory's triple stain.[2] With the exception of 3 of the gray rats, microscopic sections were made of both adrenal glands.

OBSERVATIONS

Although the adrenal glands of younger rats were also examined, medullary hyperplastic changes were seen only in the glands of the old animals, 700 days or more of age. They were found in the glands of 13 of the male and 3 of the female rats. The weights of the adrenal glands of these animals, with estimations of the degrees of hyperplasia, are listed in the table. The weights of the left and the right gland separately were known for 1 rat only (2s ♂). However, it may be seen that in many instances the growths were sufficiently large to increase the weights of the glands materially.

Hyperplasia of the medulla was either nodular or diffuse, and ranged in extent from small nests of cells (+) to complete, or almost complete,

1. Yeakel, E. H.: To be published.

2. Most of the sectioned material was prepared by Dr. Eugene Cutuly, who withdrew from the program for the study of aging to enter the armed forces.

involvement of the medulla (+ + +). Only minimal hyperplasia was found in the adrenal glands of the gray rats.

Minimal hyperplasia occurred as more or less well defined nodules within the medullary tissue, distinguished only by cytoplasm that stained more deeply than that of the surrounding cells. Similar but larger nodules tended to protrude into the cortex and to distort the normal contour of the medulla. Slight dilatation of the sinusoids of the nodule was usually seen. Larger growths took the form of nodular hyperplasia surrounding an enlarged vascular channel the walls of which were frequently thickened. The tissue of the circumscribed nodule was hyperchromatic, and was either compactly arranged or separated into distinct strands and nests of cells by dilated sinusoids. The largest growths consisted, again, of a relatively solid mass of tissue or of one broken up

The Weights of the Adrenal Gland That Showed Hyperplasia of the Medulla (Sum of Weights Both Glands)

Rat	Sex	Age, Days	Adrenal Weight, Gm.	Abnormal Glands	Degree of Hyperplasia *
410	♂	903	0.1918	One	+++
398	♂	946	0.0790	One	+++
473	♂	1,057	0.1651	Both	+++
2 S	♂	1,024	0.1396	One	+++
			0.0606	One	++
60	♂	949	0.0725	One	++
127	♂♀	903	0.0867	Both	++
830	♂	700	0.0485	Both	++
4 S	♂♀	1,006	0.0834	Both	++
999	♂	1,011	0.0383	One	+(+)
387	♂♀	901	0.0860	One	+
828	♂	700	0.0385	One	(+)
90	♂	900	0.0440	Both	(+)
954	♂♀	900	0.0473	Both	(+)
5 †	♂♀	1,112	0.0881	One ‡	+
17 †	♂	1,057	0.0810	Both	+
60 †	♂	1,055	0.1114	One ‡	(+)

* The degree of hyperplasia ranged from presence of small nests of cells (+) to complete or almost complete involvement of the medulla (+++).
† The rat was of the gray Norway strain.
‡ Only one gland was examined.

into cords of cells by sinusoidal dilatation. The mass encroached on the cortex and compressed or eliminated it. In 2 instances invasion of the capsule was observed.

The following descriptions of the gross and the microscopic appearance of individual glands are presented in a descending order of degree of hyperplasia.

410 ♂. The right adrenal gland of this rat appeared to be normal grossly and microscopically. The left on dissection was enlarged, discolored and hemorrhagic, with a cream-colored lump at the upper pole. The left adrenal vein was wide and tortuous. Microscopic sections of this gland revealed that the cortex was almost completely eliminated, the bulk of the gland being made up of tissue that resembled normal medulla but contained enlarged sinusoids (fig. 1). Some of this tissue lay outside the capsule, comprising the cream-colored protrusion noted on dissection. The cells of the growth contained clear vesicular nuclei and were uniformly stained. The sinusoids were lined with a delicate endothelium.

398 ♂   The right adrenal gland was small and of normal microscopic appearance. The left gland was enlarged, and the medulla resembled that of 410 ♂, just described.

473 ♂.   Grossly, both adrenal glands were discolored and irregularly shaped, with a long protrusion at one pole of the left gland. Microscopic sections (fig. 2) showed a greater amount of cortical tissue than was observed in the adrenal gland of 410 ♂, but the same type of disorganization of the medullary tissue. However, unlike the first 2 glands described, much of the lining of the sinusoids was hypertrophic, and several areas of fibrosis were present in the medulla. The protrusion of the left adrenal gland was composed of less loosely arranged medullary tissue.

In contrast to the glands already described, the right adrenal gland of this rat (fig. 3) contained a large, bilobed medulla made up of solid tissue, with few vascular spaces. Occasional heavy strands of connective tissue were seen. Cells located on the periphery of both lobes were stained yellow (Zenker fixation, Mallory stain), but those in the interior were pink.

2 S ♂.   Except for a narrow rim of cortical tissue a few cells in depth, the entire right gland was made up of hyperplastic medulla. The cells were solidly packed, and those at the periphery of the growth were yellow (Zenker, Mallory) as in the right adrenal gland of 473 ♂. Blood channels with thickened lining were present in the interior but were not especially prominent.

In the glands just described, no normal medullary tissue was found. The remainder of the adrenal glands contained varying amounts of normal and hyperplastic medulla.

60 ♂.   Microscopically, one adrenal gland of this rat was normal, but the bulk of the medulla of the other gland was composed of tissue that was separated into distinct cords by large vascular spaces, which were lined with heavy strands of tissue stained blue with Mallory's triple dyes (fig. 4). Grossly, the gland had appeared to be normal. In the hyperplastic region the cytoplasm of the cells was deeply stained, most heavily in the center of the mass. Adjacent to this area there was a triangular cap of medullary tissue that was normal (fig. 5). The cells here were compactly arranged, their cytoplasm was faintly stained and granular, and the nuclei were smaller and more chromatic than those seen in the hyperplastic area.

127 ♀.   In both adrenal glands a portion of the medulla was of normal appearance and a part was similar to the hyperplastic medulla of 60 ♂.

830 ♂.   The adrenal glands appeared to be normal grossly. Microscopically each gland showed a well circumscribed area of more heavily stained cells lying eccentrically in the normal medullary tissue. In one gland the nodule surrounded a dilated blood vessel, the walls of which were thickened (fig. 6). The cells were closely packed. In the other gland the cells were loosely arranged in cords and nests; capillary endothelium was normal in appearance.

4 S ♂.   Within the normal medulla of the right gland there was a large eccentric mass of fairly solid tissue surrounding a large blood sinusoid, the lining of which was thickened. With Mallory's stain, the outer cells of the mass were yellow. There were also patches of hyperchromatic cells, like those of the nodule, within the normal medullary tissue.

The medulla of the left gland of this rat was enlarged. In one area the tissue was hyperplastic and solidly arranged, and bulged into the cortex; in another region normal medullary tissue was seen. Enlarged vascular channels were present in
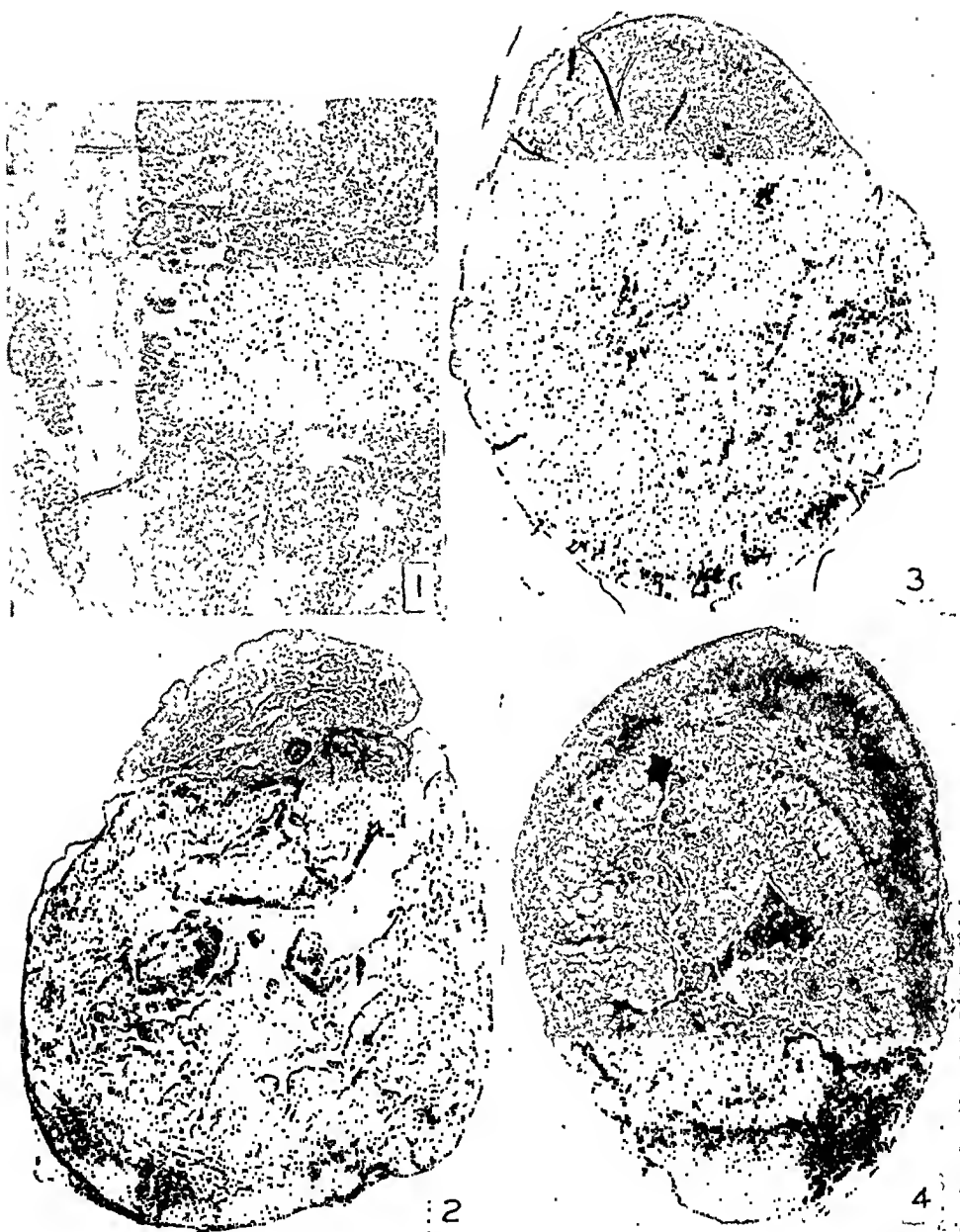
Fig. 1. (410 ♂)—Hyperplastic medullary tissue. On the right it extends to the capsule; on the left it is outside the capsule. Bouin fixation; hematoxylin and eosin stain; × 51.5.

Fig. 2 (473 ♂).—Cortex reduced to a narrow rim. At the top the medullary tissue is outside the capsule. There are large vascular spaces and heavily stained areas of connective tissue within the hyperplastic medulla. Bouin fixation; hematoxylin and eosin stain; × 15.5.

Fig. 3 (473 ♂).—Bilobed hyperplastic medulla with little vascularization. Zenker fixation; Mallory triple stain; × 15.5.

Fig. 4 (60 ♂).—Enlarged medulla with prominent vascular spaces. Note the small portion of normal medullary tissue at the top. Zenker fixation; Masson triple stain; × 15.5.

the interior of the medulla. Their endothelial lining was greatly thickened in places, and two endothelial pearls were noted in the hypertrophic tissue. The medullary tissue about the blood channels was stained more deeply than that of the normal region and appeared to be hyperplastic—resembling, on a small scale, the adrenal
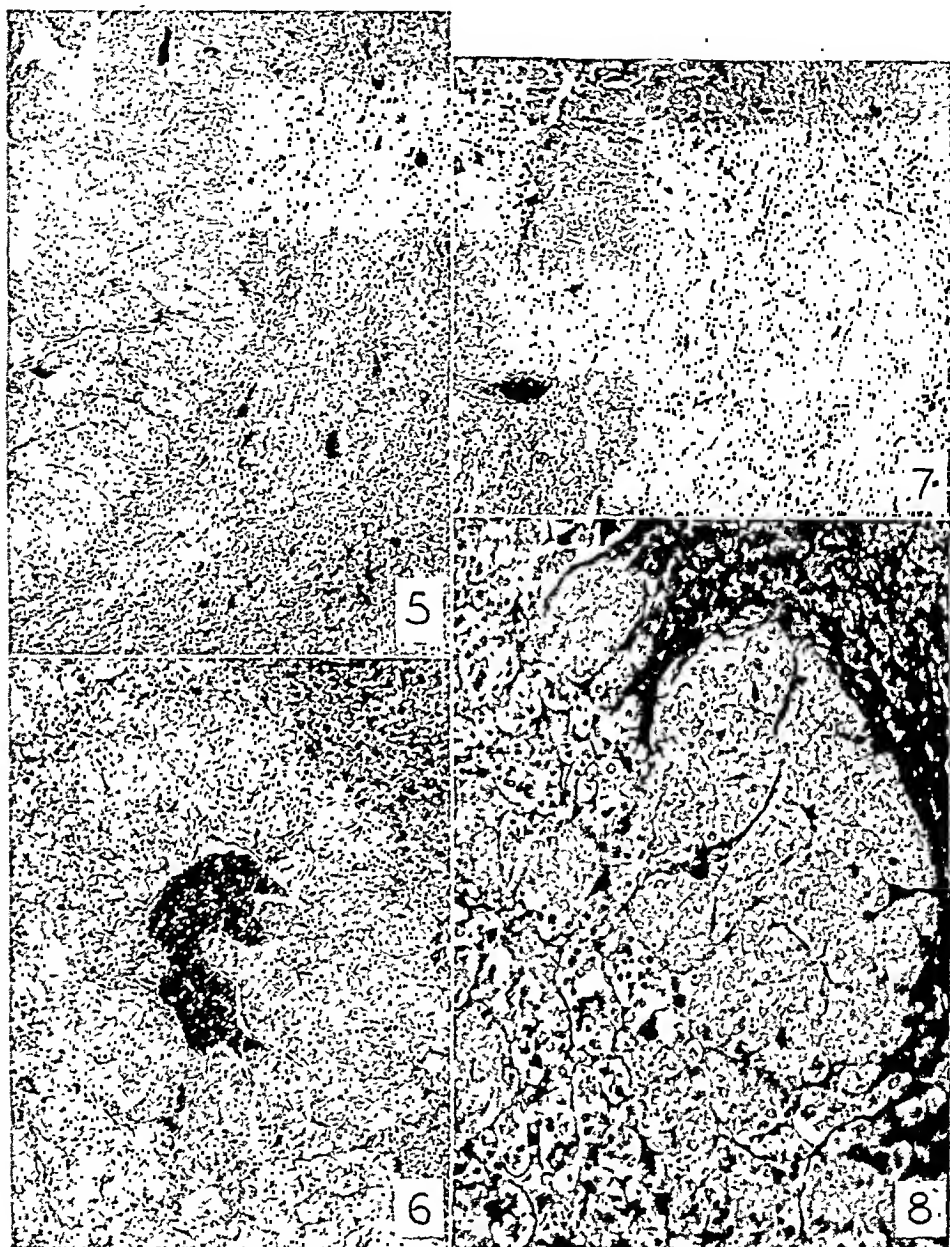
Fig. 5.—Detail of figure 4 showing (from left to right) hyperplastic medulla, normal medulla and cortex. × 71.

Fig. 6 (830 ♂).—Nodule surrounding a blood vessel. Normal medulla is seen at the bottom, to the left; cortex, at the right. Zenker fixation; hematoxylin and eosin stain; × 53.

Fig. 7 (387 ♀).—Two hyperplastic areas protruding from the medulla at the left. Bouin fixation; hematoxylin and eosin stain; × 53.

Fig. 8 (17 G ♂).—High magnification of a small nodule. Normal medullary tissue is seen on the left; cortex (dark stain), on the right. Zenker fixation; Mallory triple stain; × 141.5.

gland of 473 ♂ page 73). There was a gradual transition between the normal region, the solid hyperplastic area and the loosely arranged tissue.

2 S ♂. The right adrenal gland of this rat was described on page 5. The medulla of the left gland was enlarged and distorted in shape. Part of it was composed of a solid mass which extended to the edge of the gland and compressed the cortex into a strand of a few cells' thickness. Another portion was made up of fairly compact tissue surrounding an enlarged vascular channel. The lining was greatly thickened, and occasional epithelial pearls were seen in it. A third region of the medulla appeared to be normal histologically, with cytoplasm that took a lighter stain than the other two areas.

999 ♂. The adrenal glands were thought to be normal on dissection. Microscopic sections of the medulla of one gland showed a fairly well circumscribed nodule surrounding a blood vessel. The sinusoids of the nodule were slightly dilated. The cells resembled those of the normal portion of the medulla except for the fact that the cytoplasm of the former (with Mallory's triple stain) was deep pink, while the normal cells were faint salmon or yellow. Patches of similar hyperchromatic cells were scattered throughout the medulla.

387 ♀. In the medulla of one adrenal gland were nodules and groups of cells with homogeneously stained cytoplasm that took the dye more readily than the surrounding tissue. They resembled the patches described in the medulla of 999 ♂ ; but some projected into the cortex, giving the medulla an irregular outline (fig. 7). Slight but definite dilatation of the sinusoids was present. Elsewhere in the serial sections of this gland several sinusoids were found to be greatly enlarged and filled with erythrocytes. The endothelium was normal in appearance.

Similar aggregates of prominent hyperchromatic cells were found in the medullas of 828 ♂, 90 ♂ and 954 ♂. In the adrenal glands of the 6 gray Norway rats that were examined, only minimal hyperplastic areas of this type were seen. A nodule in the adrenal gland of a 1,000 day old male gray is shown in figure 8.

COMMENT

The degrees of hyperplasia described suggest the possibility that they may represent stages in the development of a neoplasm. Ewing stated that "in many situations adenomatoid hyperplasia becomes progressive and passes into a neoplastic process,"[3a] and referring to struma adrenalis, affecting medulla as well as cortex, he remarked that "between the nodular hypertrophies and true adenomas there appears to be every transition."[3b]

In glands in which the medullary hyperplasia had advanced beyond a minimal stage it was associated with an unusual dilatation of one or more sinusoids. Ewing wrote that while "some adenomas . . . represent chiefly an excessive response to functional stimulus, others appear to owe their existence to minor anatomical disturbances in the blood supply."[3a]

Probably identical changes were described by Staemmler[4] as adenoma of the adrenal medulla of rats with chronic nicotine poisoning. He

3. Ewing, J.: (*a*) Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1942, p. 508; (*b*) p. 512.

4. Staemmler, M.: Virchows Arch. f. path. Anat. **295**:366, 1935.

attributed the appearance of the growths to the repeated stimulation of the medullas by the daily subcutaneous injection of nicotine. In a paper dealing with medullary hypertrophy following suppression of thyroid secretion induced with thiouracil, Marine and Baumann [5] reported an enlarged medulla observed in a female rat over a year old, the description of which resembles the extensive hyperplasia seen in 410 ♂ and 398 ♂ (page 73).

No experimental procedures were carried out on the rats used in this study of aging. Nevertheless, the work of Staemmler [4] and of Marine and Baumann [5] suggests that the functional state of the other endocrine glands may play a part in the development of the medullary growths and that experimental alterations of hormonal balance may simulate changes that occur spontaneously with advancing age.

Findings reported here point to a sex difference in the frequency of the medullary hyperplasia. Of the 36 glands from 19 old female rats of both strains that were examined, 4 (11 per cent) showed some degree of medullary growth; these changes were found in 3 rats (16 per cent). Eighteen of the 35 glands from old males exhibited hyperplasia, including all of the most advanced stages. The growths were found in 13 of the 18 males (72 per cent); only 5 rats possessed adrenal glands with no morphologic abnormalities of the medullary tissue.

SUMMARY

Adrenal glands of 16 female and 15 male albino rats and of 3 male and 3 female gray Norway rats, 700 days or older, were examined microscopically. Both glands of the albino rats were studied, but only 9 from the gray rats. Hyperplasia of the medulla was observed in 13 of the male rats (72 per cent) and in 3 of the female rats (16 per cent). Of 36 glands from females, hyperplastic changes were found in 4 (11 per cent); of 35 glands from male rats, the changes were seen in 18 (51 per cent).

Varying degrees of hyperplasia were seen, and when arranged in order of size they suggested the development of an adenoma. The minimal stage consisted of a knot of hyperchromatic cells within the normal medulla. Sinusoidal dilatation was usually present, separating the strands of the medullary cords of the nodule. Similar but larger nodules surrounded enlarged blood channels. More advanced growths were made up either of relatively solid hyperplastic tissue or of tissue separated into cords by greatly dilated sinusoids or of both. In the most advanced stages the cortex was compressed to a narrow rim or was completely obliterated, with no normal medullary tissue being identified. In 2 instances invasion of the capsule was observed.

5. Marine, D., and Baumann, E. J.: Am. J. Physiol. **144:**69, 1945.

Case Reports

TUMOR OF CAROTID BODY TYPE ARISING IN THE MIDDLE EAR

PHILIP M. LeCOMPTE, M.D.; SHELDON C. SOMMERS, M.D., and FRANK D. LATHROP, M.D.
BOSTON

IN 1945 Rosenwasser [1] described a tumor of the middle ear and the mastoid process having the histologic characteristics of a tumor of the carotid body and suggested that it might have arisen from the glomus jugularis described by Guild.[2]

The following case is reported because of the resemblance of the· tumor to that described by Rosenwasser and because it seems probable that other similar tumors are described in the literature under different names.

REPORT OF A CASE

M. P., a 43 year old housewife, had had trouble with her left ear for ten years. Three years before admission she had a "polyp" removed. When examined at the Lahey Clinic on March 20, 1946, she had a polypoid mass of considerable size in the left aural canal. Roentgen examination of the petrous ridges and the mastoid processes showed the right side to be normal, while on the left there were sclerosis and thickening of the septums of the air cells, consistent with chronic mastoiditis. The laboratory findings were not remarkable.

Operation.—On June 15 endaural radical mastoidectomy was done on the left side by one of us (F. D. L.). The antrum was small and filled with some debris. When the middle ear was opened, considerable cellular material somewhat resembling a polyp and suggesting tumor tissue was seen in the region of the orifice of the eustachian tube just anterior to the promontory, eroding the intratympanic portion of the fallopian canal. Profuse bleeding was encountered, apparently arterial, and the patient lost about 1,000 cc. of blood before it could be controlled. She had mild postoperative facial paralysis, but made a good recovery. There was no sign of recurrence one year after operation.

Pathologic Observations.—The material received consisted of about 0.5 cc. of soft grayish red tissue. Microscopically, this material consisted in part of fairly dense fibrous tissue and in part of tumor tissue composed of nests of epithelioid cells surrounded by a fairly delicate fibrous and vascular stroma—a histologic picture typical of carotid body tumor (*A* of figure). The fundamental and characteristic pattern was clearly brought out by silver impregnation (*B* of figure). There was only slight variation in nuclear size in the epithelioid cells, and no mitotic figures were observed.

Both the tumor reported by Rosenwasser and the one described here appeared to have the characteristic structure of tumors arising

---

1. Rosenwasser, H.: Arch. Otolaryng. **41**:64, 1945.

2. Guild, S. R.: Anat. Rec. (supp. 2) **79**:28, 1941.
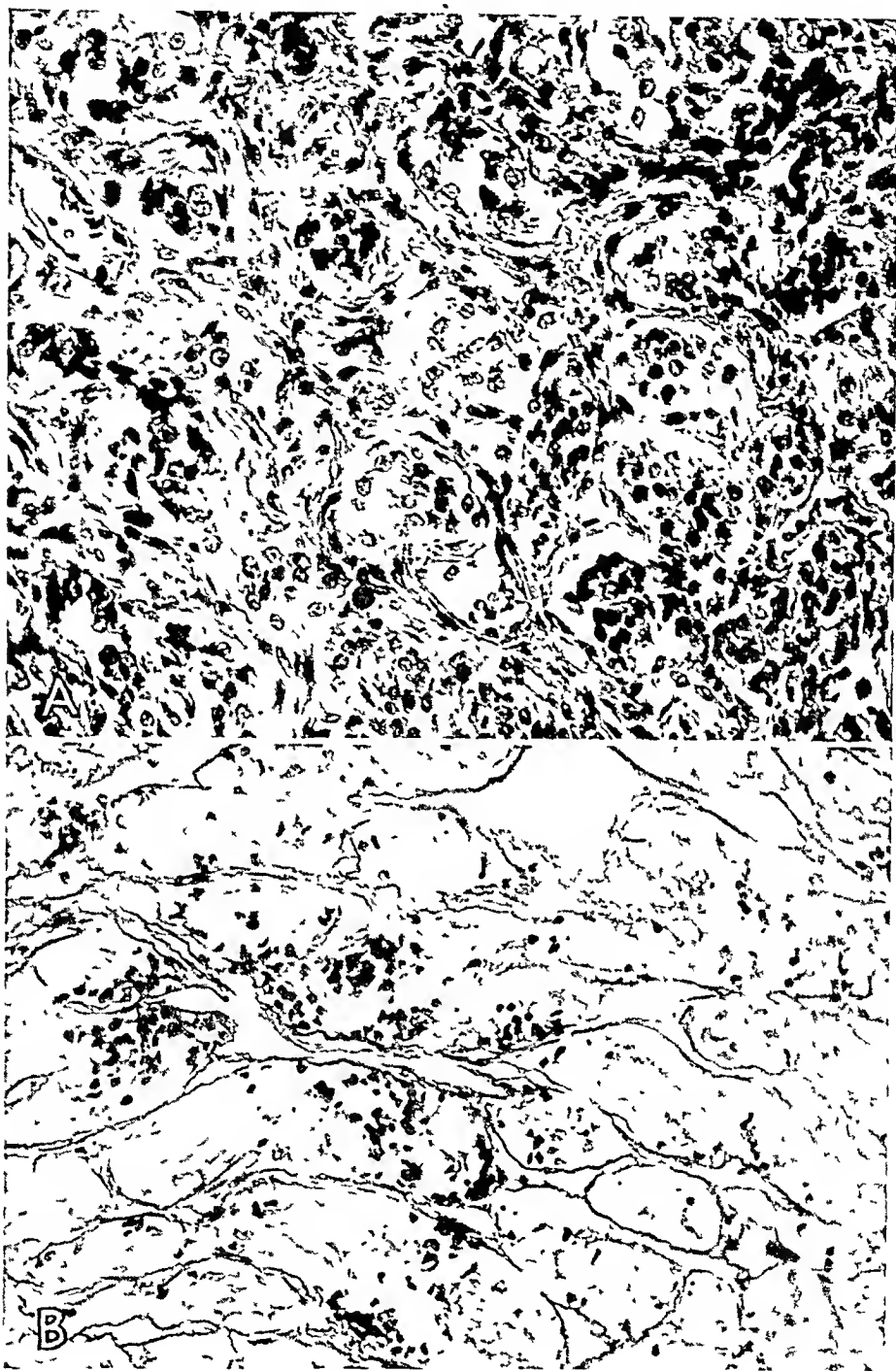
Sections of a tumor of the carotid body type that arose in the middle ear: *A*, eosin–methylene blue stain; × 300. *B*, Wilder silver impregnation; × 300.

in the carotid body as described elsewhere.[3]   In neither of these 2 cases was there any evidence of a tumor in the region of the carotid body.   Therefore, the suggestion of Rosenwasser that such a tumor might arise in the histologically similar glomus jugularis of Guild[2] seems to be a sound one.   The latter structure is said to be situated either in the adventitia of the jugular bulb, immediately below the floor of the tympanic cavity, or in the canal that transmits the ramus tympanicus of the glossopharyngeal nerve.   Guild stated that the innervation and the blood supply of this body come from the same sources that supply the carotid body, namely, the glossopharyngeal nerve and the ascending pharyngeal artery.   The suggestion is thus a reasonable one, and if future investigations disclose that the glomus jugularis functions as a chemoreceptor, the analogy could be considered complete.

The literature apparently contains no reports of other cases of tumors of the middle ear resembling tumor of the carotid body.   However, the report of Capps[4] of 2 middle ear tumors diagnosed as hemangio-endothelioma is accompanied by photomicrographs which suggest that these tumors may have been of the same type as that reported here. (Carotid body tumors, may, of course, resemble endothelioma or "perithelioma" and are so classified by some writers.)   It is interesting that in all 4 cases, i.e., those of Capps, that of Rosenwasser and the one reported here, symptoms (usually deafness) had been present for at least ten years.   Capps described the tumor as arising from the inner tympanic wall in his case; Rosenwasser, from the hypotympanum.

Several other cases of tumor of the middle ear reported as instances of endothelioma, hemangioma or hemangioendothelioma are to be found recorded in the literature.   In some of these[5] no photographs of the tumor are given, and it is impossible to judge from the description whether the structure might have resembled a carotid body tumor.[6] In a few others[7] the illustrated histologic aspect of the tumor is distinctly not that of the carotid body.

3. LeCompte, P. M.: Am. J. Path., to be published. Bloom, F.: Arch. Path. **36**:1, 1943. Gratiot, J. H.: Internat. Abstr. Surg. **77**:177, 1943.

4. Capps, F. C. W.: J. Laryng. & Otol. **59**:342, 1944.

5. (*a*) Marx, H., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1926, vol. 12, pp. 490-568. (*b*) Figi, F. A., and Hempstead, B. E.: Arch. Otolaryng. **37**:149, 1943. (*c*) Schall, L. A.: ibid. **22**:548, 1935. (*d*) Scott, S.: J. Laryng. & Otol. **54**:601, 1939. (*e*) Beck, J. C.: Illinois M. J. **9**:137, 1906. (*f*) Bryant, W. S.: Ann Otol., Rhin. & Laryng. **16**:301, 1907. (*g*) Calabresi, A.: Osp. maggiore **7**:17, 1919. (*h*) Urbantschitsch, E.: Ztschr. f. Ohrenh. **67**:365, 1913.

6. Dr. F. A. Figi stated (personal communication) that a review of the 7 tumors reported by him and Hempstead[5b] as hemangioendothelioma does not permit reclassification of any of them as of carotid body type. Through the courtesy of Dr. L. A. Schall, we have been able to examine slides of the two tumors reported by him[5c] as hemangioendothelioma; they do not have the structure of carotid body tumors.

7. Sullivan, J. A.: Arch. Otolaryng. **20**:61, 1934. Fischer, J.: Ztschr. f. Hals-, Nasen- u. Ohrenh. **5**:221, 1923. Bronzini, A.: Arch. ital. di otol. **40**:590, 1929. Jones, J. A.: J. Laryng. & Otol. **45**:265, 1930. Specht and Völker: Arch. f. Ohren-, Nasen- u. Kehlkopfh. **120**:93, 1929.

In a discussion of granular cell myoblastoma, Horn and Stout[8] reported two middle ear tumors which they described as having an "organoid" structure. Their figure 2 bears a strong resemblance to a carotid body tumor, and the question may be raised as to whether the tumor illustrated could not be so classified.

Probably most of the polypoid tissue masses removed from middle ears and mastoid processes are not examined histologically. If this were done more often, it seems likely that a small percentage would fall into the category here described.

SUMMARY

A case of tumor of carotid body type apparently arising in the middle ear is described. A possible origin from the glomus jugularis of Guild as proposed by Rosenwasser is considered. It is suggested that other tumors of this type may be recorded in the literature under different names, such as "hemangioendothelioma" and "myoblastoma."

8. Horn, R. C., Jr., and Stout, A. P.: Surg., Gynec. & Obst. **76**:315, 1943.

ARTERIOSCLEROSIS IN INFANCY

MAJOR WELLAND A. HAUSE and CAPTAIN GUNNARD J. ANTELL
Medical Corps, Army of the United States

THERE have been numerous reports on juvenile arteriosclerosis. Generally, this means arteriosclerosis of persons below the age of 20 years. The number of cases in which the patient was less than 1 year of age is small. Zeek[1] lists 5 such cases up to 1930. Scheidegger[2] reported a case in which the patient was a 7 month old infant. Brown and Richter[3] listed 4 additional cases from the literature and added 1 case of their own. Stryker[4] classified coronary occlusion of infants and children under medial calcification with fibroblastic proliferation of the intima. He recorded 15 cases, 7 of which had not been previously included in a review, and added 4 of his own. Since this condition is apparently rare, the report of an additional case seems worth while.

REPORT OF A CASE

A boy was born Sept. 10, 1946, at full term, after an uneventful labor; he weighed a little more than 7 pounds (3,175 Gm.), had a good color and a good cry and showed no abnormalities on the usual examination. The mother was 21 years old and in good health. She had had severe vomiting throughout pregnancy. At four months' gestation she was told she had renal disease and was given a salt-poor diet and her intake of fluids restricted. At six months' gestation she began to have moderately severe edema of the ankles, feet, legs and hips and of the hands. Prior to delivery she was informed that her renal disease had cleared up. Throughout pregnancy the mother drank a quart of milk daily but had no additional vitamin therapy. The maternal grandmother is living at the age of 54 but has arteriosclerosis and a "bad heart."

The baby was breast fed until one week prior to death. Beginning at six weeks, he was given daily 1 teaspoon of cod liver oil fortified with percomorph liver oil (850 U. S. P. units of vitamin D per gram). The feeding of orange juice, cereal and strained foods were as usual for the age. The development was normal, and the habits were good. On close questioning, the father stated that the baby had always seemed pale in comparison with others of the same age, and the mother stated that she had changed the baby's diapers about thirty times a day.

The baby appeared to be in good health until midnight of the day before death, Dec. 31, 1946, when he became "fussy." He vomited his feeding at 7 a. m. and became cyanotic and dyspneic for a short time. A second episode of cyanosis

1. Zeek, P.: Arch. Path. **10**:417, 1930.
2. Scheidegger: Frankfurt. Ztschr. f. Path. **54**:442, 1940.
3. Brown, C. E., and Richter, I. M.: Arch. Path. **31**:449, 1941.
4. Stryker, W. A.: Am. J. Dis. Child. **71**:280, 1946.

and dyspnea followed the 11 a. m. feeding, and this continued until admission to the hospital on Jan. 1, 1947.

On admission his temperature was 98.2 F. (by rectum); the respiratory rate was 88 and the pulse rate approximately 140. He was obviously in great distress, with pallor and cyanosis of the finger tips and lips. There was suprasternal and subcostal retraction at inspiration, and decreased breath sounds over the base of the right lung posteriorly, with tubular breath sounds over the apex. The heart was of normal size, with a rate of approximately 140 per minute, and no murmurs were heard.

The blood showed erythrocytes 3,500,000, hemoglobin 11 Gm., leukocytes 14,900, with a differential count of neutrophils 55 per cent and lymphocytes 45 per cent. A roentgenogram of the chest showed a mottled nodular infiltration throughout both lung fields with somewhat greater concentration toward the hilar areas and a slight blunting of the left costophrenic angle.

Oxygen was given by nasal catheter and penicillin by intramuscular injection, and hypodermoclysis was performed. The baby's condition did not improve and he died on January 2.

The clinical impression was: acute, severe bronchopneumonia or fulminating miliary tuberculosis.

Postmortem Examination (five hours after death).—The body measured 53 cm. in crown-heel length and weighed 6.5 Kg. No sternal abnormalities were noted. The peritoneal cavity contained no excess fluid, and the abdomen generally showed no abnormalities. The right pleural cavity contained 45 cc. of clear straw-colored fluid with an occasional fleck of fibrin; the left pleural cavity, 35 cc. of similar fluid. The pericardial sac contained 7 cc. of clear straw-colored fluid. The pleural and pericardial surfaces were smooth and shiny. The transcardiac diameter was 7 cm.; the transthoracic diameter, 11 cm. The right lung weighed 81 Gm. and the left 71 Gm. Both lungs showed decreased crepitation and were mottled light and dark red. On section these areas appeared to be due to congestion. In the posterior portion of the lower lobe of each lung were several grayish red, non-crepitant areas having the appearance of atelectasis. Bronchial and vascular markings appeared somewhat more prominent than usual. The heart weighed 50 Gm. (normal, 27) and showed moderate dilatation of the right auricle and ventricle. The muscle was somewhat pale but appeared about normal in tone. The right ventricular wall measured up to 3 mm. in thickness; the left ventricular wall, up to 10 mm. The leaflets of the tricuspid valve were short, and the occlusal margins were thickened and showed many slightly rounded, moderately red nodules measuring 0.5 to 1 mm. The leaflets of the mitral valve showed similar but more marked changes. The chordae tendineae of both the ventricles appeared shortened. The appearance was that of a moderate insufficiency of the mitral and tricuspid valves. The coronary arteries were prominent and tortuous and on section showed patchy calcification with narrowing of the lumens up to 25 per cent of normal. The aorta and the pulmonary artery and the remainder of the organs showed no gross change; the thymus weighed 20 Gm., the spleen 11 Gm., the liver 245 Gm., the adrenal glands together 6 Gm. and the kidneys together 52 Gm.

Microscopic Examination.—Section of the mitral valve showed the occlusal margins of the leaflets to be somewhat irregular, with small masses of fibrous connective tissue adherent to one surface; no vegetations or ectatic blood vessels were seen. In the papillary muscle of the left ventricle there were several areas where there was necrosis of a number of muscle cells, with beginning deposition of calcium in some of them. Surrounding the necrotic areas there were a few polymorphonuclear leukocytes (fig. 1 *A*). In the wall of the left ventricle, especially in the subendocardial region, there were several various-sized linear areas of

necrosis; the muscle fibers were eosinophilic and finely granular and had lost their striations; the nuclei were pale or not seen. Some pyknotic nuclear debris was seen in these areas, and a few polymorphonuclear leukocytes were present in
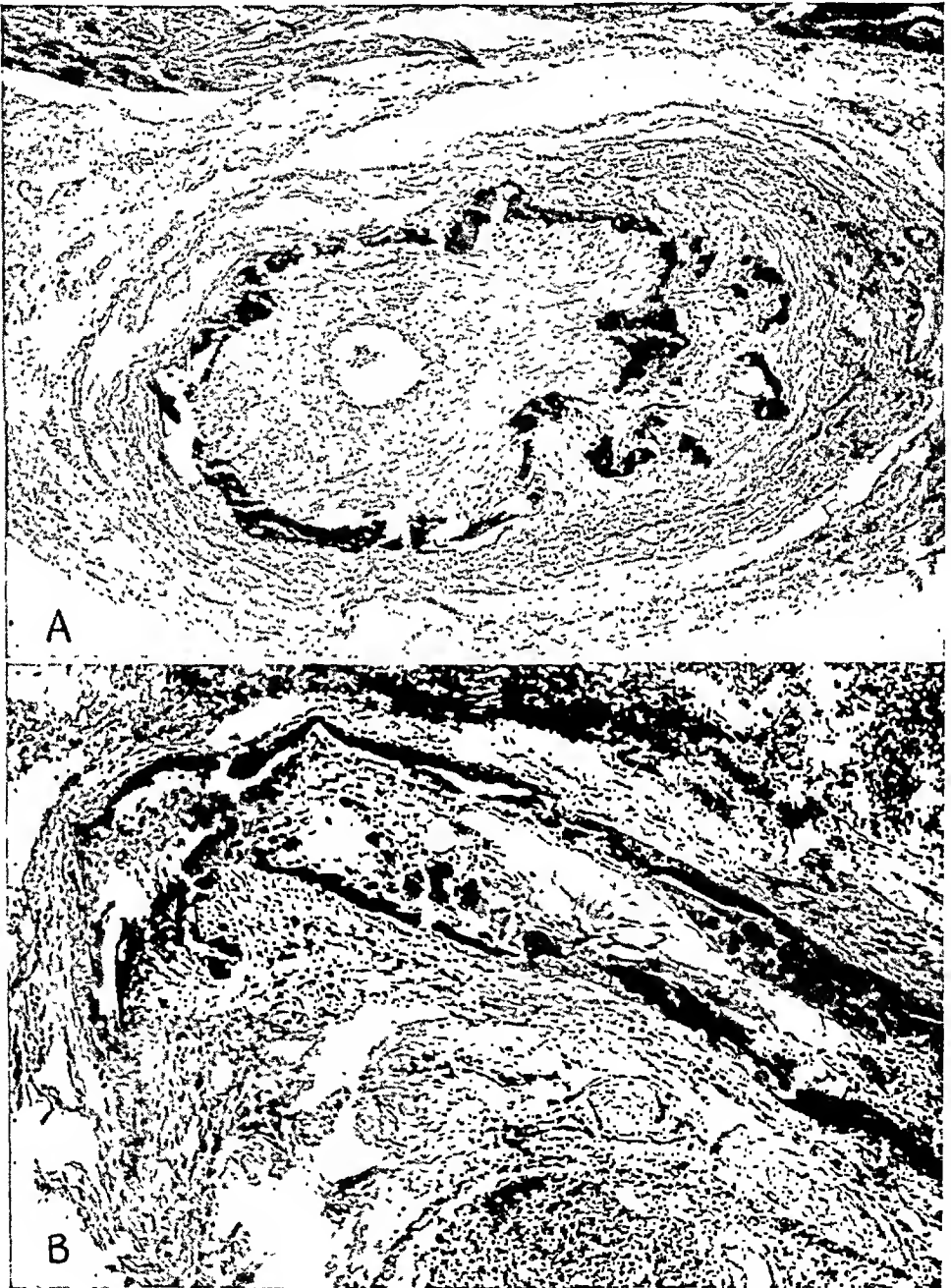
Fig. 1.—*A*, photomicrograph of the papillary muscle of the left ventricle showing a partially calcified area of necrosis; × 70. (U. S. Army Institute of Pathology Negative 187787.) *B*, photomicrograph of the coronary artery showing severe arteriosclerosis; × 70. (U. S. Army Institute of Pathology Negative 99913.)

and around them; slight hemorrhage was noted around some of the areas. The appearance was that of focal myocardial necrosis. The wall of the right ventricle showed no changes.

Sections through the various branches of the coronary arteries showed severe arteriosclerosis of the Monckeberg medial sclerosis type (fig. 1 *B*). The lumens were greatly reduced in size—from 5 to 50 per cent of normal, approximately; one branch of the anterior descending branch of the left coronary artery was completely occluded by a fairly recent thrombus, and an offshoot of the circumflex branch was completely obliterated by connective tissue. All branches showed marked proliferation of the subendothelial loose connective tissue, which often contained a few capillaries; in many areas the cells contained various-sized vacuoles, which presumably were of lipoid nature. In most of the sections the internal elastic lamina was frayed or broken but could still be identified; deposited on it was a thick irregular layer of calcium salts which partially or completely encircled the vessel and completely replaced the smooth muscle of the media—in some areas a small portion of the media was preserved. The adventitia occasion-
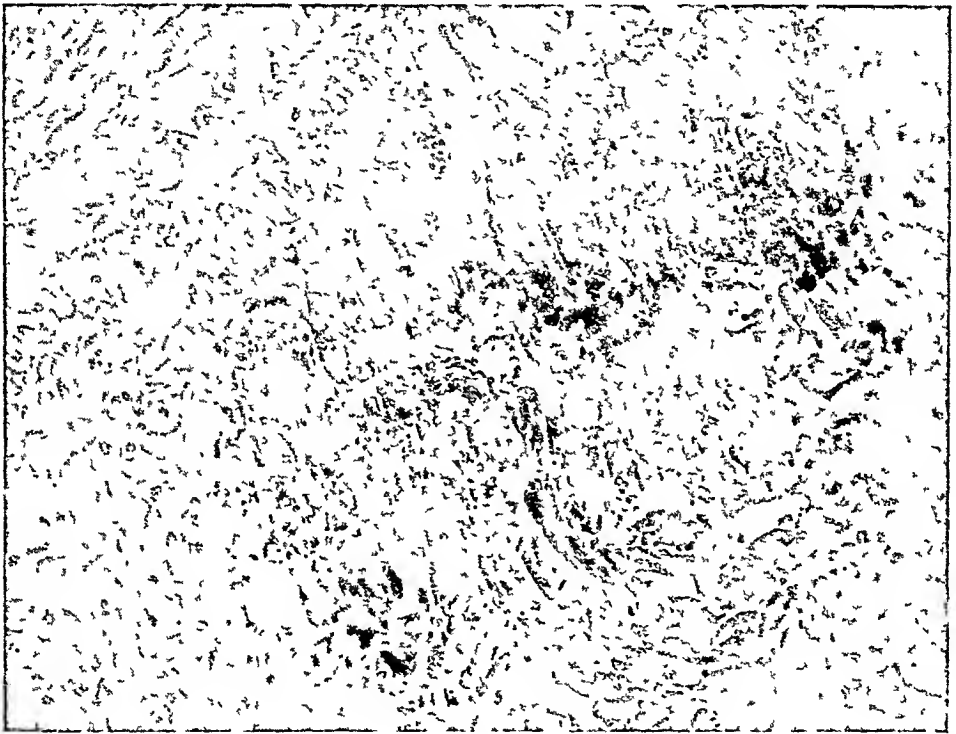
Fig. 2—Photomicrograph of a medium-sized artery in the connective tissue adjacent to a tracheobronchial lymph node, showing giant cells of the foreign body type closely approximated to the calcified internal elastic lamina; × 160   (U. S. Army Institute of Pathology Negative 99917.)

ally showed an increase in collagenous connective tissue; frequently there was spotty lymphocytic infiltration, with sometimes a few plasma cells and occasional polymorphonuclear leukocytes

Medium-sized arteries in the connective tissue adjacent to the thyroid gland, in the connective tissue adjacent to the pancreas, in the periadrenal fat, at the hilus of the spleen, in the mesentery and in the renal pelvis showed changes similar to those described in the coronary arteries. In the connective tissue adjacent to a tracheobronchial lymph node was seen a medium-sized artery cut in longitudinal section (fig. 2); it showed changes similar to those in the coronary arteries and, in addition, numerous giant cells of foreign body type in close approximation to the intimal side of the zone of calcification around the internal elastic lamina.

The lungs showed severe passive congestion with small patchy areas of atelectasis.

The other organs showed no significant abnormalities.

COMMENT

Brown and Richter [3] have discussed the etiologic possibilities of infantile arteriosclerosis; their case closely resembles that reported here. The history that possibly there was renal disease of the mother during gestation is too vague to be given much weight in an account of the development of the arteriosclerosis of the infant. The suggestion of polyuria conveyed by the mother's comment concerning excessive use of diapers had no corroboration in the gross and microscopic appearance of the kidneys. While the daily dose of vitamin D given the baby was above the optimum, such dosage is not unusual and probably has no bearing on the case. Unfortunately, no attempt was made to dissect out the parathyroid glands at autopsy, but the fact that they were not observed on total removal of the thyroid gland suggests that they were not grossly enlarged. Although no sections of bones were taken, a roentgenogram of the chest taken ante mortem showed no osseous changes, and the sternum, the costal cartilages, the ribs and the vertebrae did not show gross changes at autopsy.

SUMMARY

A case of severe arteriosclerosis of a 3½ month old infant is presented. As in the majority of recorded cases, no definite etiologic factor could be identified.

PRIMARY OVARIAN PREGNANCY

ESTHER H. DALE, M.D., DETROIT

CASES of genuine primary ovarian pregnancy are rare enough so that it is worth while to report such a case when it does occur.

REPORT OF A CASE

The patient was a 34 year old white woman who had been married for sixteen years and had one child, who was living and well. She had experienced one miscarriage, ten years previous to the present history. In November 1946 she failed to menstruate. In December she began to have hemorrhage and passed many blood clots, but observed no membranes in the material passed. Dilatation and curettement were done. Microscopic examination of the curetted material showed proliferative endometrium, but no chorionic villi or decidual cells were found in the sections. Following the curettage, bleeding continued sporadically for three weeks, then ceased spontaneously.

In January this patient consulted the physician again, complaining of a persistent dull aching sensation in the lower part of the abdomen. Laparotomy was done, with removal of the uterus, tubes and ovaries. The uterus was normal in size and appearance, and requires no further description. The tubes were somewhat congested but not more so than could be accounted for by the operative procedure. They were not appreciably enlarged, and the fimbriated end of each tube was patent. The tubes were distinctly separate from the ovaries. The left ovary was slightly enlarged and was partially cystic. The cysts contained a clear straw-colored fluid.

The right ovary was globular in shape and measured 5 cm. in diameter. Its surface was fairly smooth and showed no tears or ruptured areas. Its color was a dark reddish brown, like that of endometrial cysts, and indeed the general appearance of the organ suggested an endometrial cyst which has displaced and replaced the normal ovarian tissue. Routine sections were made of the wall of the cyst and of the hemorrhagic mass filling the lumen. Microscopic examination of these sections revealed the unmistakable presence of young chorionic villi. The embryo has not been recognized either grossly or microscopically, but serial sections were not made. However, it is well to know that, according to Curtis,[1] destruction of the ovum may occur within the follicle at an early period.

It is no part of my intention to review all the literature on the subject of ovarian pregnancy. But it would not be out of order to draw attention to a few of the salient and interesting facts in this connection.

According to Novak,[2] there are about 50 reported cases in which the diagnosis of ovarian pregnancy is based on acceptable evidence.

1. Curtis, A. H.: Obstetrics and Gynecology, Philadelphia, W. B. Saunders Company, 1933, vol. 3, pp. 386-387.

2. Novak, E.: Gynecological and Obstetrical Pathology, Philadelphia, W. B. Saunders Company, 1940.

Spiegelberg [3] formulated certain criteria which a case must fulfil if it is to be considered one of true primary ovarian pregnancy. These are as follows:

1. The tube, including the fimbriated end, must be intact and must be distinctly separate from the ovary. Norris [4] added that the tube must show no microscopic evidence of pregnancy.

2. The gestational sac must definitely occupy the normal position of the ovary.

Fig. 1.—Photomicrograph of large chorionic villi as seen in the blood mass. × 185.

3. The gestational sac must be connected with the uterus by the utero-ovarian ligament.

4. Unquestionable ovarian tissue must be demonstrable in the walls of the sac; according to Williams,[5] it must be found at several places

3. Spiegelberg, O.: Arch. f. Gynäk. **13**:73, 1878.

4. Norris, C. C.: Surg., Gynec. & Obst. **9**:123, 1909.

5. Williams, J. W.: Obstetrics, New York, D. Appleton and Company, 1903, p. 537.

in the wall, at some distance from each other. Williams felt this requirement to be necessary because in certain cases of tubal or broad ligament pregnancy the ovary may become flattened out and to a certain extent incorporated in the sac wall.

The foregoing criteria are fulfilled perfectly in the case under discussion. The first three are gross features and cannot be demonstrated now that the specimen has been cut. If any one had suspected that the specimen represented other than an ordinary endometrial cyst, it could
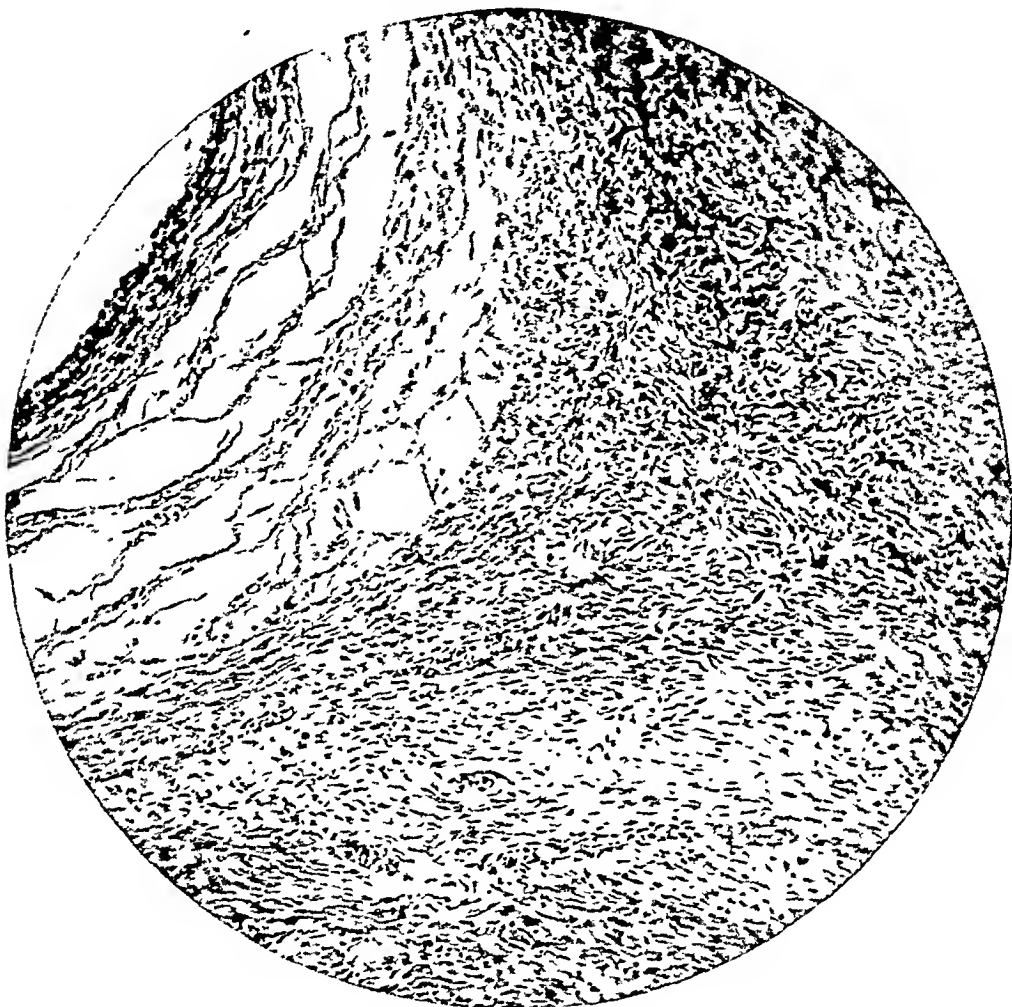
Fig. 2.—Photomicrograph showing a part of the wall of the cyst. Part of a follicular cyst is seen at one edge. × 160.

have been photographed, but this was not done. The fourth criterion is easily fulfilled, since unmistakable ovarian tissue has been seen in all parts of the sac wall, with corpora albicantia, follicular cysts, primitive follicles and, of course, the typical ovarian stroma.

By some writers,[6] ovarian pregnancies have been broadly classified into two types. In one of these the ovum is surrounded by a corpus

6. Studdiford, W. E., and Lardaro, H. H.: Am. J. Surg. **33**:566, 1936.

luteum wall or is in close relationship with a corpus luteum. In the other type no such relationship can be observed. The ovarian pregnancy reported here belongs in the second of these two categories. It is quite possible and even probable that such pregnancies have their beginning in the follicle, but the development of the trophoblast has resulted in the destruction of the lutein cells and their consequent disappearance. In some cases of ovarian pregnancy the presence of a decidual reaction has been reported. But Jordan [7] expressed the opinion that the cells which have been interpreted as decidual in these cases are in reality lutein cells of the almost destroyed corpus luteum. I was unable to observe any areas which I considered evidence of a decidual reaction.

Other more elaborate classifications of ovarian pregnancies have been set up, as for example the classification proposed by Stux.[8] He listed the following four types:

1. Intrafollicular. This is the most common form. The ovum is retained in the follicle, becomes fertilized there, implants itself and develops. Why the ovum does not escape from the ruptured follicle can be merely a matter of conjection.

2. Superficial. On rupture of the follicle, the ovum reaches the surface of the ovary, is fertilized and becomes implanted there, owing to some anatomic feature of the particular ovary, such as furrows, sulci, wrinkles, thickening of the ovarian capsule, small areas of cystic degeneration or endometriosis.

3. Interstitial. In this form nidation takes place in the interstitial part of the ovary, outside the follicle. The train of events is thought to be substantially as follows: Owing to a tough ovarian capsule, an overdistended follicle bursts laterally, and the ovum escapes into the interstitial substance of the ovary. Then a surface rupture of the same follicle also occurs, through which the sperm enters the follicle and then finds its way to the ovum lying outside of the follicle wall. This seems complicated and involved but is believed to be the explanation of those cases in which the trophoblast and the corpus luteum are found next to each other in a lateral relationship, in contrast to the fourth type which is called,

4. Suprafollicular. This is actually a form of superficial nidation. A superficial blood clot at the opening of the ruptured follicle holds the ovum at its exit, where it is fertilized, becomes implanted and develops.

The duration of an ovarian pregnancy is usually longer than that of a tubal pregnancy because the ovary is a more elastic and resilient organ than the tube; it is capable of far more distention and stretching, as may be easily seen from the large size which ovarian cysts often attain.[9] In some cases ovarian pregnancy has been known to go on to full term, with a living child. In other cases the end result has

7. Jordan, H. E.: Surg., Gynec. & Obst. **54**:485, 1932.

8. Litzenberg, J. C., in Nelson Loose Leaf Living Surgery, New York, Thos. Nelson & Sons, 1941, vol. 7, p. 536.

9. (*a*) Eckerson, E. B.: Am. J. Surg. **54**:487, 1941. (*b*) Schumann, E. A.: Extra-Uterine Pregnancy, New York, D. Appleton and Company, 1921.

been a lithopedion. In a series of 38 cases in which the pregnancy lasted for seven months or longer, there were 12 in which it resulted in a living infant. In 22 cases the mother lived, and in 8 cases both mother and baby lived.[10]

The known incidence of true primary ovarian pregnancy is low. At St. Luke's Hospital in New York, 339 cases of ectopic pregnancy included one ovarian pregnancy. This is an incidence, in this series, of 0.29 per cent.[9a]

A fair number of cases have now been reported. But since there may be early rupture of the sac or early degenerative changes without rupture, it is quite likely that some cases are not recognized as such.[11]

SUMMARY

The history and the pathologic observations in a case of true ovarian pregnancy are reported.

The criteria of true ovarian pregnancy set up by Spiegelberg and amplified by Williams and Norris are recapitulated.

Classifications of ovarian pregnancies are briefly reviewed.

10. Nicholls, R. B.: Am. J. Obst. & Gynec. 42:341, 1941.
11. Titus, E. W.: Am. J. Obst. & Gynec. 38:516, 1939.

Laboratory Methods and Technical Notes

A METHOD OF DECALCIFYING BONE FOR HISTOLOGIC SECTION

IRVING M. RICHMAN, M.D., NEW ORLEANS; MAX GELFAND, B.S., and J. M. HILL, M.D. DALLAS, TEXAS

THE CLINICIAN, as well as the pathologist, has been hampered by the abnormally long periods of time necessary for decalcification of bone and the lack of uniformity of the sections finally produced. Reports are generally confined to the acids, organic, inorganic or a combination, used as the decalcifying agents. At times other substances, such as phlorglucinol or mercury bichloride, have been added in order to lessen destruction of the tissue. Thus, Clapp [1] reported using nitric acid with phlorglucinol; McNamara [2] used a combination of nitric and trichloroacetic acids, and De Galantha [3] used nitric acid with trinitrophenol. Kramer and Shipley [4] reported on the affinity of magnesium citrate for calcium and on its use as a decalcifying agent. Similar use of citrate solutions has been reported by Shelling and Halpersohn [5] and Evans and Krajian.[6] In a review of the commonly used methods, Jaffe [7] and McClung [8] pointed out the disadvantages of the consumption of time and the destruction of tissue frequently encountered.

Attempts to speed up the decalcification of bone by increasing the concentrations of the reagents have resulted in swelling of the fibers and destruction of the cells. To minimize these untoward reactions and at the same time speed up the reaction G. H. Wilson [9] and R. A. J. Wilson [10] suggested that a vacuum be used over various acid solutions, the reaction being thereby physically speeded up. By this method the time necessary for the decalcification of some specimens had been cut to twenty-four hours. The time consumed by the other methods enumerated varied from several days to several weeks.

The need of a method both simple and reliable by which bone could be decalcified speedily without destruction of tissue led to the following investigation.

From the Department of Pathology, Baylor University Hospital Laboratory.

1. Clapp, M. P.: Am. J. M. Tech. 1:31, 1935.
2. McNamara, W. L., and others: J. Lab. & Clin. Med. 25:874, 1940.
3. De Galantha, E.: J. Tech. Methods 17:72, 1937.
4. Kramer, B., and Shipley, R.: Science 66:485, 1927.
5. Shelling, D. H., and Halpersohn, M. B.: Arch. Path. 5:835, 1928.
6. Evans, N., and Krajian, A.: Arch. Path. 10:477, 1930.
7. Jaffe, H. J.: Arch. Path. 8:817, 1929.
8. McClung, C. E.: Handbook of Microscopic Techniques, New York, Paul B. Hoeber, Inc., 1937.
9. Wilson, G. H.: J. Path. & Bact. 39:531, 1934.
10. Wilson, R. A. J.: Am. J. Clin. Path. (Tech. Supp.) 12:79, 1942.

Bone is composed of an organic matrix in which mineral salts are deposited. Mature bone is composed 50 per cent of water and as much as 24 per cent of fat. The dried fat-free material consists 30 to 40 per cent of organic matter, the remainder being a complex salt mixture. Analytic studies of the mineral composition of bone have given results that conform to the empiric formula

$$n\ Ca(PO_4).CaX$$

where n varies between 2 and 3 and X may be carbonate, oxide, fluoride, chloride or sulfate. Roentgen ray diffraction studies show the bones to have an atomic arrangement similar to that of podolite, a mineral of the apatite series.[11]

The exact method by which calcium is deposited in bone is still under investigation. It appears, however, that a vital process of the
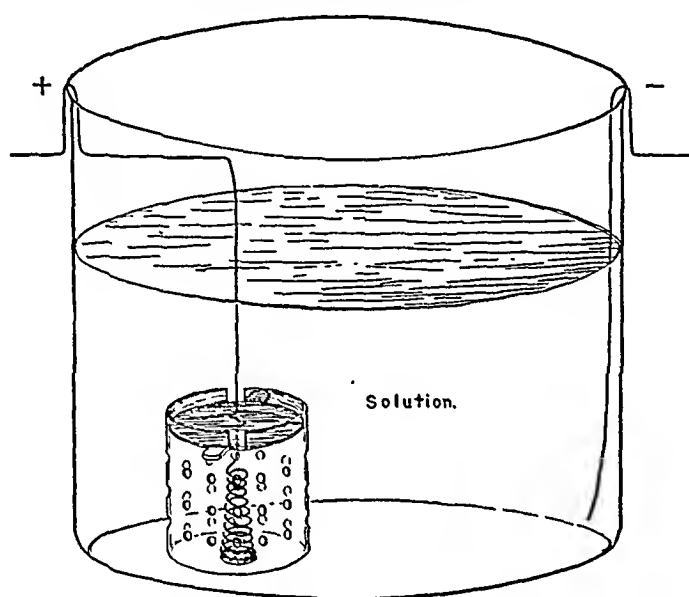
Fig. 1.—Bone container placed in the decalcifying solution. Note the arrangement of the cover and of the electrodes.

tissue is at least in part responsible. On the other hand, calcium would seem to be best removed from bone by any reaction which increases ionization of the calcium. Of the various means of speeding up this chemical reaction without cellular destruction, a process involving the following method was found to be most suitable.

DESCRIPTION OF METHOD

The devised method employs the forced migration of the positively charged calcium to a negatively charged electrode or a "deplating" of the calcium carbonate and phosphate from the bone. The apparatus used is shown in figure 1. The bone container is made of plastic material with a locking lid. Multiple holes in the container facilitate fluid exchange about the bone specimen. The platinum wire is coiled to impinge on and hold fast the bone specimen.

11. Grollman, A.: Diseases of Bone, New York, Oxford University Press, 1939.

Any source of direct current may be used. Good results have been obtained by the use of a 6 volt storage battery. A constant source is produced by the use of a rectifier attached to the regulation 110 AC circuit. With the use of a rheostat the amperage may be controlled to produce desirable results.

The vessel containing the electrolytic bath should be of glass and large enough to hold the necessary electrodes without crowding. The bath while in service
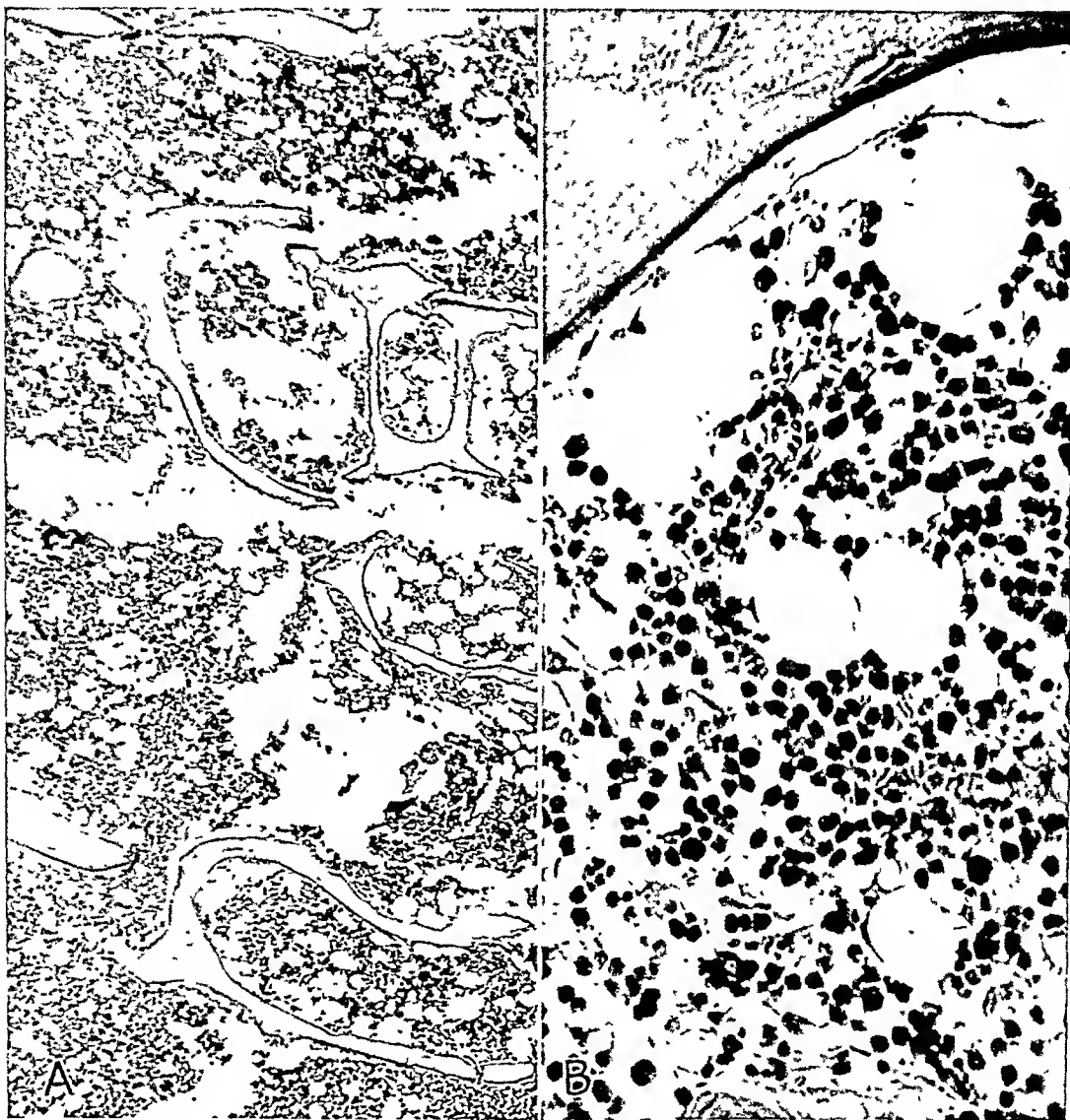
Fig. 2.—*A*, section of sternum 1 cm. in thickness decalcified in three hours with preservation of marrow. Hematoxylin and eosin stain; × 42.

B, detail of marrow cells in section shown in *A*. × 250.

should be controlled at temperatures between 30 and 45 C. Higher temperatures not only increase the speed of reaction but cause disintegration of the tissue. A large vessel lessens the chance of overheating and may be used continuously without control over the period of decalcification.

The electrodes should be made of platinum, hard carbon or tungsten. Experiments with electrodes made of iron, copper, brass and "castalloy" had shown that these metals were readily dissolved, the electrolysis having a much greater affinity for the anode than for the bone.

Any suitable reagent that can be placed into an aqueous solution which will dissociate the calcium from the carbonate and phosphate ions may be used.

Solutions of various substances that would react with the inorganic constituents of the bone have been tried. Although comparatively few of these substances were used before desirable results were obtained, the following ones suggested themselves:

(*a*) Oxidizing substances, such as chromates and nitrates. These rapidly destroyed the organic matter.

(*b*) Organic acids and their corresponding salts. These, because of their low degree of ionization and conduction, produced decalcification at a slow rate.

(*c*) Carbon dioxide in high concentrations. This retarded the rate of decalcification.

(*d*) Inorganic salts. Although producing good conduction and having some affinity for calcium they removed this element at a slow rate.

(*e*) Nonoxidizing and highly ionizable acids that produce water-soluble compounds of calcium. These acids produce good decalcification.

In an attempt to "fix" and decalcify simultaneously, a mixture of hydrochloric acid and formaldehyde was used. Very poor removal of calcium and some disintegration of tissue took place. The assumption that formic acid produced from the formaldehyde was responsible for the untoward reaction led to the use of various concentrations of hydrochloric and formic acids. The results were surprising. The best decalcification over the shortest period of time without disturbance of tissue had been obtained with a 10 per cent solution of formic acid and an 8 per cent solution of hydrochloric acid. Repeated use of 10 per cent formic acid and 8 per cent hydrochloric acid continued to produce desirable results (fig. 2). It is assumed that the formic acid softens the tissue and allows the acid reaction to take place more rapidly. Higher concentrations of formic acid and/or excess contact with the specimen produces disintegration of the tissue.

Proper decalcification of all bone samples treated with these two acids in the electrolytic bath is generally accomplished in two to six hours. Care must be taken not to allow the bone specimen to remain in the decalcification bath longer than necessary. Overtreatment, especially in the presence of heat, will bring about destruction of tissue.

Following decalcification, the bone is cleared in aniline and immediately placed in paraffin. Prior to staining, the slides are dipped once into a 1 per cent solution of lithium carbonate for adequate neutralization of the acid decalcifying agent.

SUMMARY

A very effective means for removing calcium compounds from bone with minimal injury of tissue has been shown to result from the use of an electrolytic bath with 8 per cent hydrochloric acid and 10 per cent formic acid as the electrolytic medium.

A RAPID PROCEDURE FOR THE EMBEDDING OF EYES

MARY E. CARSTEN, B.A., NEW YORK

THE ROUTINE preparation of eyes for microscopic study is a notoriously slow process. This is due to the fact that rapid methods of dehydration and embedding cause shrinkage and consequent distortion. The value of a rapid method for diagnostic purposes is obvious, especially in cases of suspected malignant tumors. Furthermore, experimental work might be retarded for weeks or months by the waiting for sections. The problem, therefore, was to find a water-soluble embedding medium whereby dehydration and consequently shrinkage would be avoided and the time of preparing sections would be shortened considerably.

Among the many synthetic products of modern chemical industry, "carbowax" compounds (solid polyethylene glycols, molecular weight above 1,000) appeared to be particularly suitable as embedding mediums because of their chemical inertness, noncorrosive nature, good thermal stability, high aqueous solubility, high molecular weight and low hygroscopicity. They do not support the growth of molds and do not deteriorate. They are manufactured in various stages of polymerization by the Carbide and Carbon Chemicals Corporation.[1] With increasing molecular weight the aqueous solubility and the hygroscopicity decrease and the melting point rises. One of the series, "carbowax 4000," has been used for preparing sections of muscle for electron microscopy [2] but cannot be used in the preparation of eyes because of the difficulties encountered in mounting the sections on slides. Since "carbowax" compounds are highly soluble in water and in aromatic hydrocarbons and, to a somewhat limited extent, in aliphatic hydrocarbons, there is left only a small choice of liquids to float the sections on. Benzine and ethers do not dissolve the compounds, but the sections become extremely brittle. Mounting sections from liquid petrolatum, another nonsolvent, proved impossible.

The difficulty was overcome by first infiltrating the specimens with a "carbowax" compound and then with another less water-soluble wax. Among several "carbowax" esters, one nearly insoluble in water, "carbowax distearate," does not infiltrate tissue directly at all, but because it is miscible with "carbowax" compounds it infiltrates specimens previously infiltrated with a "carbowax" compound. (A related substance, diethylene glycol distearate, has been used for general tissue work in an embedding mixture after preceding dehydration.[3])

The method arrived at after a large number of grades of "carbowax" compounds and "carbowax" esters (supplied by the courtesy of the Carbide and Carbon Chemicals Corporation and the Mellon Institute)

---

1. McClelland, C. P., and Bateman, R. L.: Chem. & Engin. News **23**:247, 1945.

2. Richards, A. G.; Anderson, T. F., and Hance, R. T.: Proc. Soc. Exper. Biol. & Med. **51**:148, 1942.

3. Steedman, H. F.: Nature, London **156**:121, 1945.

had been tested on more than 100 specimens—human eyes and dogs' and monkeys' eyes—is as follows:

"Carbowax 1000" and "carbowax 1540 distearate" (the numbers refer to the molecular weight) are placed in the incubator at a temperature of 56 C. and allowed to melt. To 100 cc. of each is added 0.1 Gm. of "duponol C," a wetting agent derived from technical lauryl alcohol of the series of alcohol sulfates, manufactured by E. I. du Pont de Nemours, which will dissolve completely overnight. (The wetting agent shortens the time of infiltration by half and at the same time acts as an emulsifying agent, preventing excessive crystallization of the wax on solidification. All "carbowax" compounds used in this method and referred to from now on have this wetting agent added at 0.1 per cent by volume.)

The eye, fixed in Bouin's solution for two or three days, is cut in half. The half eye is placed in a large dish with distilled water for one-half hour, the distilled water being changed every ten minutes. The specimen is now transferred to a mixture of "carbowax 1000" and distilled water, equal parts, for one-half hour at a temperature of 37 C. It is then placed into four successive changes of "carbowax 1000," remaining two hours in each, all at 37 C. The carrying over of excess wax from one solution to the next is avoided by carefully removing it with filter paper. In the last change the specimen remains overnight.

Then it is placed into a mixture of "carbowax 1000" and "carbowax 1540 distearate," equal parts, for one-half hour, and then into four successive changes of "carbowax 1540 distearate," two hours each, except for the last in which the specimen remains overnight. Since the solidification point of "carbowax 1540 distearate" is higher than that of "carbowax 1000," the temperature of the incubator has to be raised to 45 C. The specimen, cut surface up, is embedded the next morning in fresh "carbowax 1540 distearate" in a paper boat such as that used for paraffin embedding. It is hardened slowly (to avoid excessive crystallization of the wax), the embedding boat being placed in the incubator at 37 C. until evening and on top of the incubator overnight.

The block is then mounted on a metal carrier block in the same manner as the paraffin block. Sections are cut with a rotary microtome. The edge of the knife should be parallel to the cornea-nerve axis of the eye. Sections can be cut as thin as 7 microns. These, however, are difficult to mount; the preferable thickness is 10 to 15 microns. The sections come off in ribbons, which are placed on paper. The ribbons are cut and the sections floated singly on a saturated aqueous solution of sucrose till they straighten out,[4] and are immediately mounted on slides. (In pure water the wax would slowly dissolve and mounting of the sections be difficult.) Excess sugar solution is drained off from the slides with small pieces of filter paper, which are left on the slides while these are drying. The slides are placed in the incubator at 37 C. for at least two hours in order to make the sections stick to the slides.

Now they are placed in dehydrated alcohol, then in alcohol and ether, equal parts, then in a 3 per cent solution of celloidin (a concentrated preparation of

---

4. The exact timing according to the thickness of the sections is vital in order to obtain good sections without folds. The sections should be removed just before all the folds have straightened out, as this process will continue in the incubator until the sugar solution dries. Even thick sections should not remain in the sugar solution more than two minutes, and it is preferable to add a few drops of water to the Petri dish which contains the sugar solution.

pyroxylin) in alcohol and ether, each for two minutes. Thereafter they are put in a vertical position to allow the celloidin to dry on them while the excess celloidin drains off. After five minutes they are transferred to a saturated solution of lithium carbonate in 70 per cent alcohol, in which the celloidin will harden. As soon as the sections have lost their yellow color, the celloidin cover is removed from the backs of the slides, and the slides are placed in distilled water. The celloidin comes off the slides as the sugar dissolves, and the sections stick firmly to the celloidin membrane. The celloidin is trimmed around the sections, which can immediately be stained exactly as any celloidin section is, or can be left in distilled water overnight and stained the next day.

Routine stains, such as hematoxylin-eosin, Masson's trichrome, Weigert's elastic stain, the Van Gieson stain and the Loyez or Spielmeyer myelin stain, can be applied. The sections are cleared in carbolxylene or in xylene and mounted on the celloidin membrane in "clarite" or synthetic resin.
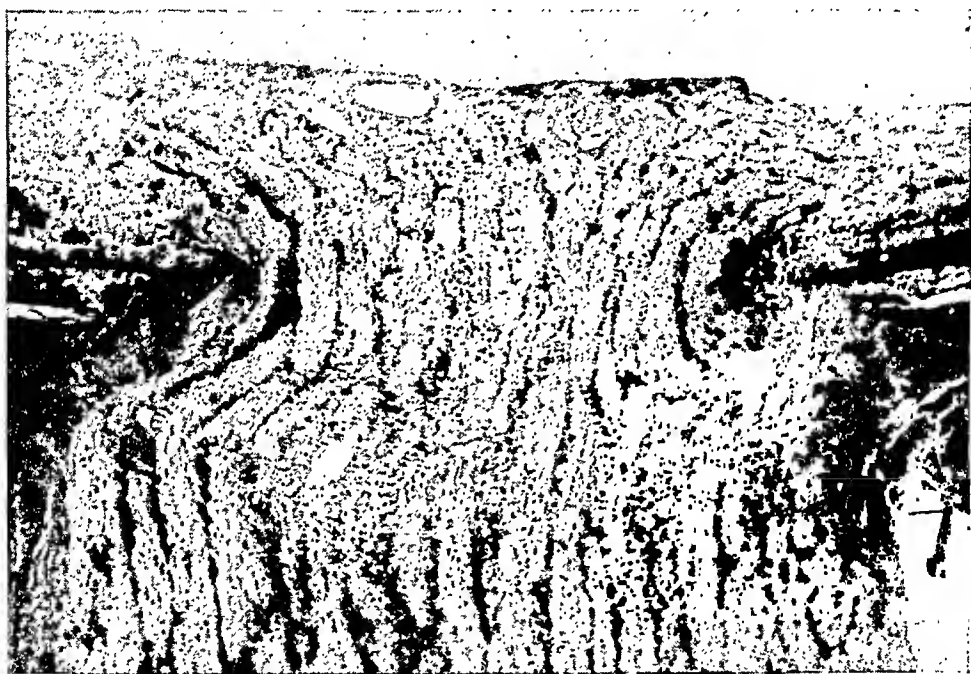
Fig. 1.—Human optic nerve head; hematoxylin-eosin; × 55.

The first two changes of "carbowax 1000" should be discarded after use; the last two can be used as the first two for the next specimen. Of the "carbowax 1540 distearate," only the first change has to be discarded; the others may be used as successively lower changes. All bottles containing "carbowax" compounds should be stoppered, as these compounds are slightly hygroscopic.

A specimen can be kept in a 4 per cent solution of formaldehyde after having been fixed in Bouin's solution, when embedding right after fixation is not desired. Good results have also been obtained by putting the specimens after fixation into 50 per cent alcohol for two days and then into 70 per cent alcohol up to the time of embedding.

The sugar solution can be filtered and used again.

Sections should not be kept on slides longer than twenty-four hours, because the crust of sugar forming on the slides will eventually disrupt the sections. They

should be removed from the slides by way of passing them through celloidin as already described and can then be kept in 70 per cent alcohol for storage. Blocks should be stored in a dry place after covering the cut surface with a thin layer of molten "carbowax 1540 distearate."

The described method does not produce any shrinkage of the specimens, there is no fungous growth and the tissue is well preserved (fig. 1). Regarding the lens, the same difficulty is encountered as in using the paraffin method, namely, brittleness and poor infiltration.

In a hematoxylin-eosin stain the cells of the layers of the retina. especially the ganglion cells, stain distinctly (fig. 2). In contrast to

Fig. 2.—Retina of monkey; hematoxylin-eosin; × 200.

ordinary paraffin or celloidin sections, myelinated nerve fibers will stand out clearly in a hematoxylin-eosin stain. As there is no contact of the specimen with myelin solvents like xylene or chloroform, and the contact with alcohol-ether after mounting is exceedingly short, the myelin is not dissolved, and no special myelin stain is necessary. Special myelin stains, however, turn out well too, whether the Loyez or the Spielmeyer method is used.

The main advantage of the new method lies in the possibility of obtaining sections of whole eyes as thin as paraffin sections within four days after fixation, while the routine procedures require three and a half weeks in paraffin and five months in celloidin as used in this laboratory. Furthermore, by eliminating contact with alcohol and other

organic solvents, it avoids shrinkage and possible detrimental effects in some staining methods.

The method might be adapted for use in other fields of pathology. It might be applied to nerve tissue because of good myelin stains and especially to spongy tumors which fall apart when cut on the freezing microtome and cannot be stained well with myelin stains after paraffin embedding.

SUMMARY

Eyes can be embedded rapidly, with minimal shrinkage and distortion, by means of water-soluble "carbowax" (polyethylene glycol) and "carbowax" ester, used in succession. The entire process of embedding, sectioning and staining can be accomplished in four days.

Books Received

GYNECOLOGY, WITH A SECTION ON FEMALE UROLOGY. By Lawrence R. Wharton, M.D., assistant professor of gynecology, Johns Hopkins University School of Medicine; assistant attending gynecologist, Johns Hopkins Hospital; consultant in gynecology, Union Memorial Hospital, Hospital for the Women of Maryland, Sinai Hospital and Church Home and Infirmary. Second edition. Pp. 1,027, with 480 illustrations. Price $10. Philadelphia and London: W. B. Saunders Company, 1947.

The first edition was issued in 1943. The section on embryology and that on congenital malformations have been rewritten, and changes and additions have been made, especially in the discusions of physiologic and chemotherapeutic matters. Recent advances in operative technic are·described and illustrated. The part on urology has been enlarged. The text is well organized and clearly written. The descriptions of the morphologic characteristics of the organs in question and of their diseases are accurate and will be of great interest to the pathologist. The illustrations, many of them borrowed from classic sources, are uniformly excellent. The last chapter of the book deals with irradiation as used in the practice of gynecology and female urology, but the technical details of radiotherapeutic methods are not discussed. Should not the clinician be as interested in the details of those methods as in the details of other methods of treatment? The book maintains fully the high standards of the Hopkins school of gynecology.

SURGICAL PATHOLOGY. By William Boyd, M.D., M.R.C.P., F.R.C.P., professor of pathology, University of Toronto, Canada. Sixth edition. Pp. 858, with 530 illustrations, including 22 colored figures. Price $10. Philadelphia and London: W. B. Saunders Company, 1947.

Since 1925 a new edition of this book has been published every four or five years, each with changes and additions to keep abreast with advances in pathology. In the present edition one important topic is the pathologic anatomy and physiology of the congenital heart diseases, in the treatment of which surgery is making notable progress. The results of the continued efforts to deal adequately with all topics in pathology of special surgical interest are evident in all parts of the book. Of topics still worthy of consideration may be mentioned the pathology of the nasal sinuses, transfusion, and the surgical relations of blood grouping. The section on biopsy as employed in the diagnosis of cancer should be revised and brought up to date.

RADICAL SURGERY IN ADVANCED ABDOMINAL CANCER. By Alexander Brunschwig, M.D., professor of surgery, University of Chicago. Pp. 324, with 118 illustrations. Price $7.50. Chicago: University of Chicago Press, 1947.

This book deals with the author's methods of radical surgery and his results in 100 cases of advanced abdominal cancer. The details—clinical, pathologic, surgical—of this remarkable series are clearly described and well illustrated. Special emphasis is placed on the value of newer methods of supportive treatment. The book marks a great advance in its field.

THE PATHOLOGY OF TRAUMATIC INJURY: A GENERAL REVIEW. By James V. Wilson, M.D., M.R.C.P., Major R.A.M.C. (T), pathologist to Harrogate and District General Hospital and to the Royal Bath Hospital, Harrogate. Foreword by Philip H. Mitchiner, M.D., M.S., F.R.C.S., surgeon to St. Thomas Hospital. Pp. 192, with 61 illustrations. Price $6. Baltimore: Williams & Wilkins Company, 1946.

This monograph reviews the literature on the pathology of trauma to the human body in the light of the author's personal work and experiences in war. The first part takes up traumatic shock, the pathologic aspects of burns, crush injury—traumatic anuria, fat embolism, blast injury, the pathologic aspects of wounds and wound infection. The second part has chapters on injuries of the chest, the blood vessels, the abdomen, the nervous system, the bones and the joints. The illustrations, mostly borrowed from other writers, are well chosen and instructive. The book is a good review of the pathology of trauma and will be of valuable service to clinicians, pathologists and students.

DISEASES OF THE CHEST: WITH EMPHASIS ON X-RAY DIAGNOSIS. By Eli H. Rubin, M.D., F.A.C.P., F.C.C.P., attending physician, Division of Pulmonary Diseases, Montefiore Hospital and Country Sanatorium, New York; visiting physician in tuberculosis and physician-in-charge, Chest Clinic, Morrisania City Hospital, New York. The Principles of Surgical Treatment. By Morris Rubin, M.D., assistant visiting surgeon, Triboro Hospital and Morrisania City Hospital, New York. Pp. 685, with 355 illustrations (24 in color). Price $12. Philadelphia and London: W. B. Saunders Company, 1947.

The first section takes up anatomy and physiology as applied to diseases of the chest, and roentgenology. The second section discusses acute and chronic forms of pneumonia, with a chapter on sulfonamide compounds and antibiotics. The third section is devoted to pulmonary tuberculosis. The chronic diseases in which the bronchi are primarily concerned are the subjects of section 4, and section 5 deals with diseases of the mediastinum, the pleura and the diaphragm. Section 6 is devoted to the surgical treatment of the diseases considered in the book, which is well illustrated with roentgenograms.

MEMORANDA ON MEDICAL DISEASES IN TROPICAL AND SUB-TROPICAL AREAS. Eighth edition. Pp. 396, illustrated. Price 7 s. 6 d. London: His Majesty's Stationery Office, 1946.

This book, sponsored by the British War Office, has been carefully revised and brought into line with the advances of knowledge during the 1939-1945 war. In a form convenient for medical officers it contains some 60 or more well written and concise memoranda on the essentials of the more important tropical diseases, with instructive illustrations in the text and on plates.

COLOR ATLAS OF HEMATOLOGY: WITH BRIEF CLINICAL DESCRIPTIONS OF VARIOUS DISEASES. By Roy R. Kracke, M.D., dean and professor of clinical medicine, Medical College of Alabama, Birmingham, Ala. For Medical Students, Laboratory Technicians and General Practitioners of Medicine, with Clinical and Hematologic Descriptions of Blood Diseases, Including a Section on Terminology, a Section on Technic and a Summary of Blood Findings in Various Diseases. Pp. 204, illustrated with 32 plates in full color and 3 plates in black and white. Price $5. Philadelphia, London and Montreal: J. B. Lippincott Company, 1947.

CARDIAC ANOXIA AS THE FACTOR DETERMINING THE OCCURRENCE OF EXPERIMENTAL VIRAL CARDITIS

JOHN MUSSER PEARCE, M.D.

AND

GERTRUDE LANGE, A.B.

BROOKLYN

PREVIOUS reports [1] have dealt with the experimental demonstration that a variety of filtrable viruses capable of infecting the rabbit will at times produce inflammation in the heart after being inoculated at a peripheral site such as the skin and subcutaneous tissues, the testis or the upper respiratory tract. The predominant lesion is myocarditis, but not uncommonly this is accompanied with valvulitis, endocarditis or pericarditis. In those instances in which virus III or pseudorabies virus was used as the infecting agent, the lesions revealed the characteristic intranuclear inclusion bodies indicating the presence of the virus. The types of viral inflammation that are not characterized by inclusion-bearing cells can be proved to be of viral cause by the ability of a saline suspension of a heart containing such a lesion to infect another animal.

Although the occurrence and the nature of the cardiac lesion are in themselves of interest, of even greater significance is the fact that the incidence and the severity of these lesions were remarkably increased not only by the grossly crude and unphysiologic procedure of cardiac puncture but also by the intravenous injection of a solution of acacia or of an extract containing betahypophamine ("pitressin") [1a] made at the time of inoculation of virus. There are several hypotheses that might explain the mechanism by which two such physically and pharmacologically dissimilar substances bring about cardiac localization of virus. The work described here substantiates one of these—the hypothesis that cardiac anoxia is the responsible factor—and fails to support others. The experiments by which the anoxia hypothesis has been tested, the methods used in them, and their results are described in the main body of the paper. The great bulk of negative experimentation that has dis-

1. (a) Pearce, J. M.: Arch. Path. **28**:827, 1939; (b) **34**:319, 1942. (c) Pearce, J. M., and Levine, H. D.: Am. Heart J. **25**:102, 1943.

proved other conceivable explanations for cardiac localization of virus under these same conditions is described in less, detail in the terminal discussion.

Both substances, betahypophamine by a constriction of the coronary arteries [2] and acacia by an interference with the gaseous interchange between the hemoglobin of the red blood cells and the surrounding environment,[3] produce anoxia of the heart. If anoxia is the factor that determines the localization of virus in the heart and the consequent production of viral myocarditis or endocarditis, the administration of other drugs having an ischemic or anoxic action on the heart should result in a similar localization of peripherally inoculated virus and a corresponding inflammation of muscle, valve or pericardium. If, on the contrary, anoxia is of no localizing significance or is only one of many factors of "heart strain," "heart damage" or "overburdening" of the heart, the subjection of the experimental animal to other procedures known to affect the action of the heart or to be toxic to it or to damage the myocardium in some way other than by ischemia should bring about the localization of virus in the heart and the production there of lesions of viral origin.

Following this line of reasoning, we performed the first series of experiments described in the following pages. Rabbits inoculated peripherally with virus underwent further treatment that may be divided into two categories, the one designed to produce cardiac anoxia, and the other to affect the heart but not to cause ischemia. The preliminary localizing procedure and the incidence of cardiac lesions have then been correlated.

FIRST SERIES OF EXPERIMENTS

Method and Material.—Virus III[4] was the infecting agent used in all but a few of the experiments. This virus is highly suitable for histologic studies, since lesions caused by it constantly have in them cells containing easily recognizable large acidophilic intranuclear inclusion bodies typical of those classified by Cowdry as type A.[5] These inclusions identify the lesion, their occurrence indicating the presence of virus. It has the additional advantage that few rabbits from the available breeding stock are insusceptible to it[6] and inoculation of active virus

2. (*a*) Clark, G. A.: J. Physiol. **68**:166, 1929. (*b*) Essex, H. E.; Wegria, R. G. E.; Herrick, J. F., and Mann, F. C.: Am. Heart J. **19**:554, 1940. (*c*) Sollmann, T.: A Manual of Pharmacology, Philadelphia, W. B. Saunders Company, 1942, p. 459.

3. Christie, A.; Phatak, N. M., and Olney, M. B.: Proc. Soc. Exper. Biol. & Med. **32**:670, 1935. Studdiford, W. E.: Surg., Gynec. & Obst. **64**:772, 1937. Raven, R. W.: Brit. M. J. **1**:950, 1940.

4. Rivers, T. M., and Tillett, W. S.: J. Exper. Med. **38**:673, 1923. Miller, C. P., Jr.; Andrewes, C. H., and Swift, H. F.: ibid. **40**:773, 1924. McCartney, J. E.: Annual Report of the Metropolitan Asylums Board, London, 1924-1925, p. 152.

5. Cowdry, E. V.: Arch. Path. **10**:23, 1930.

6. Pearce, J. M.: Proc. Soc. Exper. Biol. & Med. **38**:872, 1938.

can be expected to be followed almost invariably by disease. On the other hand, its virulence is so low that premature experimental deaths do not occur. In order to indicate that the phenomenon of cardiac localization is not a characteristic of virus III only, in 5 of the 88 experiments vaccine virus was used and in 6 the myxoma virus.[7]

The inoculum was a saline suspension of an infected rabbit testis that was removed aseptically from an animal killed on the fourth or fifth day after intra-testicular injection of a similar virus suspension. The suspension was prepared as follows: The infected testis was ground in a mortar with sterile sand and 0.85 per cent sodium chloride solution. The resulting paste was diluted to make a 5 to 10 per cent suspension, and the supernatant fluid was used for inoculation.

All the rabbits were young males weighing between 2,000 and 3,500 Gm., the majority weighing about 2,500 Gm. No attempt was made to select them according to breed or color, however, and they were obtained from several sources.

To infect the 88 experimental animals, 0.5 to 2 cc. of virus suspension was injected into each testis. Immediately thereafter groups of 6 each were given intravenously a solution of either digitalis, papaverine hydrochloride, nikethamide, corn syrup or 0.85 per cent sodium chloride. Another group of 6 was subjected to narcosis induced by inhalation of chloroform. A group of 5 was given barium chloride intravenously, a group of 7 epinephrine hydrochloride and groups of 20 each acacia and "pitressin."

Fifty-six rabbits inoculated with virus but otherwise untreated served as controls. The results from these animals were collected during the previous studies as well as in the experiments described here.

The digitalis was administered in the form of "cedilanid"[8] in doses of 1.0 to 1.7 mg. per kilogram. To 3 of the 6 animals in this group a second dose was given four days after the first.

Papaverine hydrochloride was injected at the rate of 2 mg. per kilogram. Two animals received repeated doses. The powdered drug was dissolved in saline solution so that 1 cc. of solution contained 2 mg.

The dose of nikethamide was 45 mg. per kilogram. That this was an adequate amount to produce an effect was indicated by the fact that 2 of the animals went into convulsions and all had obvious acceleration of heart rate and respiration.

The corn syrup[9] was diluted by adding an equal amount of 0.85 per cent sodium chloride, and 50 cc. of this 50 per cent solution was injected into the marginal vein of the ear as rapidly as possible.

---

7. The vaccine virus was a highly virulent strain isolated from a spontaneously infected rabbit by one of us (J. M. P.) in 1938 (J. Infect. Dis. **66**:130, 1940). The myxoma virus was procured from Dr. Richard Shope, of the Rockefeller Institute. It was the strain originally obtained from Dr. A. Moses of the Oswaldo Cruz Institute in Brazil and used by both J. R. Hobb (Am. J. Hyg. **8**:800, 1928) and T. M. Rivers (J. Exper. Med. **51**:965, 1930) and maintained by Dr. Shope in the laboratories of the Rockefeller Institute in Princeton since 1933. The strain of virus III that was used has been carried in rabbits by testicular passage in this laboratory for the past ten years. It was given to us originally by Dr. Carl TenBroeck, of the Rockefeller Institute, and was isolated originally by Dr. T. M. Rivers (J. Exper. Med. **38**:673, 1923).

8. "Cedilanid" is a brand of lanatoside C, an initial glucoside obtained from Digitalis lanata, and manufactured by the Sandoz Chemical Works, Inc., New York.

9. "Karo syrup," manufactured by Corn Products Refining Company, Argo, Ill., and Kansas City, Mo.

The chloroform was administered by inhalation from a leather cone in the open room air in quantities sufficient to produce continuous unconsciousness for sixty to one hundred minutes.

The 0.85 per cent solution of sodium chloride was injected as rapidly as the solution could be forced from a 50 cc. syringe through a no. 20 gage hollow needle into the marginal vein of the ear. A total of 100 cc. was administered.

A 1 per cent solution of barium chloride was prepared from the chemically pure reagent. Isotonic solution of sodium chloride was then added to a calculated dose of 10 mg. per kilogram, to this solution, to make a total volume of 10 cc.

The epinephrine hydrochloride was injected at the rate of 0.01 mg. per kilogram per minute during a period of two to eight minutes.

Solutions of acacia were made by dissolving the dried powder in warm 0.85 per cent saline solution in amounts sufficient to make a concentration of 20 per cent. This solution was injected intravenously as rapidly as possible in doses of approximately 50 cc.

Betahypophamine was given intravenously either in a single injection of 0.5 cc. of "pitressin" at the time of inoculation of the virus or in several doses of this drug variously spaced throughout the experimental period.

TABLE 1.—*Data on Rabbits Inoculated with Virus and Thereafter Submitted to Procedures Tending to Cause Cardiac Anoxia*

Factors Causing Myocardial Anoxia	Animals Treated	Animals with Cardiac Lesions Caused by Virus	
		Number	Percentage
Barium chloride *	5	5	100
Epinephrine hydrochloride *	7	6	85.7
Betahypophamine † *	20	10	50
Acacia *	20	18	90

* Solutions of the substances were administered by intravenous injection.
† This was administered in the form of "pitressin."

In all groups all the rabbits were killed three to six days after inoculation and autopsies made immediately. The examination always included histologic observation of the testis and the heart. Sections of tissue fixed in Zenker's solution or in a 10 per cent solution of formaldehyde were stained routinely with hematoxylin and eosin and on occasion with Giemsa stain. The hearts were examined externally in the gross but were not opened, since it had been found previously [1a] that sections more satisfactory for microscopic study could be obtained by fixing the intact organ and later trimming it and embedding it in paraffin in such a way that all four chambers and one or more valves were included in the section in their usual relationships. By cutting several parallel blocks from a single heart it was not difficult to show at least three of the valves and often four without resorting to serial section.

Results.—The four procedures described in the foregoing section which reduce the oxygen supplied to the heart were followed by a high incidence and a high degree of severity of viral lesions of the heart. As shown in table 1, carditis resulted from the intratesticular inoculation of virus when this was accompanied with one or another of these procedures, as follows: in 100 per cent of the animals given barium chloride, in 85.7 per cent of those given epinephrine hydrochloride, in

50 per cent of those given betahypophamine and in 90 per cent of those given acacia. All the other procedures were followed by an incidence and an intensity of lesions comparable to those in the controls. In no group did more than 16.7 per cent of the experimental animals show cardiac lesions, and these were all slight in extent and comparable to those seen in the 16.1 per cent of the 56 untreated animals that exhibited myocardial or endocardial viral lesions. These figures are listed in table 2.

A detailed description of the character, the localization and the severity of the lesion of the heart does not come within the scope of this paper. There are, however, two general observations that bear on the more immediate problem of the cardiac localization of peripherally introduced virus. Probably the more significant of these is the observation that the lesions which develop after acacia has been injected into

TABLE 2.—*Data on Rabbits Inoculated with Virus and Thereafter Submitted to Procedures That May Affect the Heart But Do Not Cause Myocardial Anoxia*

Factors Affecting Heart	Animals Treated	Animals with Cardiac Lesions Caused by Virus	
		Number	Percentage
100 cc. of saline solution *	6	0	0
Corn syrup *	6	1	16.7
Nikethamide *	6	0	0
Digitalis *	6	0	0
Chloroform narcosis	6	1	16.7
Papaverine hydrochloride *	6	1	16.7
Controls: virus inoculations only	56	9	16.1

* The substances were administered intravenously.

the venous blood stream are not only predominantly of the right ventricle but not uncommonly confined to the right side of the heart, while those that follow the injection of drugs that cause constriction of the coronary arteries are chiefly in the left ventricle and especially in the inner half of the myocardium and the large papillary muscles. Of less importance but certainly worthy of note in that it also supports the theory of cardiac anoxia is the observation that there is a quantitative difference in the severity of the lesions in the two large groups. Those that occurred in the small number of animals that were not submitted to procedures causing cardiac anoxia were considerably milder and less widespread than those in the anoxic group, being in general of the same order of severity as those in the nontreated controls.

COMMENT ON FIRST SERIES

The anatomic distribution of lesions between the two ventricles furnishes secondary evidence that a lack of oxygen is of importance in the development of viral lesions of the heart, since it is exactly the

distribution of the areas to which the flow of oxygen is diminished by the different actions of the drugs constricting the coronary arteries and acacia. Thus in the case of constriction of the coronary arteries the right ventricle and the auricles are spared because the muscular walls of these chambers are so thin in the rabbit and contain so many endothelium-lined crevices communicating with the cavities that they are less dependent on coronary circulation and may receive a major part of their oxygen and nutriment directly from the blood within them. The thick left ventricle, however, is supplied by the blood flowing through the coronary arteries, and when that flow is diminished the muscle suffers. The inner portions of the myocardium and the plump papillary muscles of the left ventricle receive the terminal branches of the arteries and are therefore the regions most severely affected by the narrowing of the lumens of these vessels. This narrowing obviously may be brought about by the active contraction of the smooth muscle of the arterial walls that occurs under betahypophamine[2] or barium chloride stimulation[10]; or it may result from the great increase in the intramyocardial pressure that occurs, as well as the increase in intracardiac pressure, during the period of increased contractile force and acceleration of rate[11] brought about by epinephrine. The elevation of arterial blood pressure that follows the administration of all three substances also causes the intramyocardial pressure to rise. In vivo experiments conducted in this laboratory by our colleagues Johnson and Di Palma[12] have demonstrated that during systole the intramyocardial pressure of the left ventricle exceeds the intra-aortic and hence the intracoronary pressure. The coronary arteries are thereby compressed and the volume of blood flowing through them to the inner part of the myocardium and the papillary muscles is diminished. As was indicated earlier by Anrep and his associates,[13] in strongly beating hearts the volume of blood flowing through even the more superficial arteries is reduced by the increase of intramyocardial pressure brought about by the stronger contraction.

The entirely different mechanism by which acacia produces cardiac anoxia explains why the lesions are predominantly on the right side of the heart when an intravenous injection of this substance is combined with the inoculation of virus. The acacia interferes with the interchange of oxygen between the hemoglobin of the red blood cells and the sur-

---

10. Sollmann,[2c] p. 554.

11. Goodman, L., and Gilman, A.: The Pharmacological Basis of Therapeutics, New York, The Macmillan Company, 1941, p. 403.

12. Johnson, J. R., and Di Palma, J. R.: Am. J. Physiol. **125**:234, 1939.

13. Anrep, G. V.; Cruickshank, E. W. H.; Downing, A. C., and Subba Rau, A.: Heart **14**:111, 1927. Anrep, G. V., and Hausler, H.: J. Physiol. **65**:357, 1928. Anrep, G. V.; Davis, J. C., and Volhard, E.: ibid. **73**:405, 1931. Anrep, G. V., and von Saalfedd, E.: ibid. **79**:317, 1933.

rounding fluids and tissues. This action has been well demonstrated by Christie, Phatak and Olney [2] and others and has been used to explain the cases of accidental death that followed the use of this substance as a plasma substitute some years ago. The greatest dilution of blood and hence the greatest interference with the passing of oxygen from red blood cell to tissue fluid occurs in the right auricle and ventricle as the acacia pours in from the injection site, the marginal vein of the ear, through the superior vena cava. Not only is the blood reaching the left ventricle more highly oxygenated after passing through the lungs, but also the mixture of blood of the superior and inferior venae cavae has become more complete and has decreased the local concentration of acacia and thereby diminished the interference with gaseous interchange.

Those procedures which were followed by the appearance of viral lesions of the heart have been placed in table 1, while those which only occasionally brought about cardiac localization of virus have been placed in table 2. Those in table 1 also have in common their ability to produce myocardial anoxia and therefore bear out the original hypothesis that it is the deficiency of oxygen which determines the occurrence of the viral carditis. Those in table 2, although they are capable of damaging the heart or altering its function in a variety of ways, do not tend directly or indirectly to decrease the oxygen available to that organ.

Other hypotheses that might be put forward to explain the cardiac localization of viral lesions follow the somewhat indefinite concept of "overburdening" or "straining" the heart and thereby decreasing its resistance to the localization of the small amounts of circulating virus that presumably had entered the blood stream at the site and the time of inoculation. According to this reasoning, the sudden addition of a large quantity of hypertonic solution of acacia to the venous blood might so increase its volume and thereby the venous blood pressure as to cause acute dilatation of the right auricle and ventricle and transient failure of the right side of the heart. The prevalence of the lesion of the right ventricle as compared with that of the left when acacia is used as the localizing agent is in line with this explanation. That it is not the true explanation has been shown by two types of experiments. In one of these, measurements of the blood pressure of the right carotid artery and of that of the marginal vein of the left ear were made and recorded by a kymograph before, during and after the period in which acacia or isotonic solution of sodium chloride was injected into the marginal vein of the right ear of the rabbit. With the doses used in the work tabulated here, no significant alteration occurred in either pressure. In the other type of experiment direct observations of the heart of the intact anesthetized animal were made while acacia was injected into the aural vein. No dilatation of the right ventricle could be made out. However, pallor of the right ventricle and auricle appeared almost immediately after

the injection was started and persisted until the inflow of solution of acacia was stopped.

Electrocardiograms [14] taken during the injection of acacia solution have failed to show any change in the recorded activity of the heart other than that in the rate of contraction. Slight acceleration was the rule, but occasionally slowing occurred.
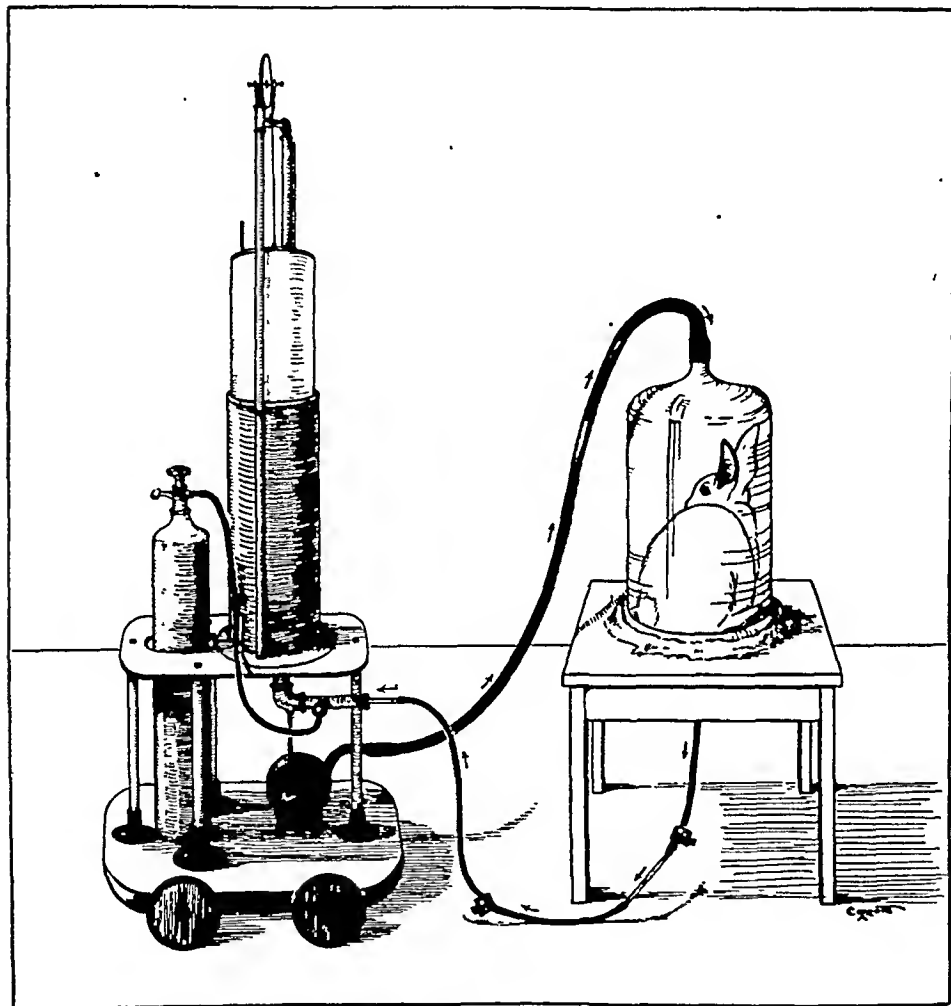
Apparatus combining a bell jar sealed with "plasticine," a spirometer and a nitrogen cylinder by which rabbits were subjected to an atmosphere deficient in oxygen.

Although the interpretation of the results of these experiments on the basis of cardiac anoxia is convincing it rests to a large extent on indirect evidence. Therefore, in order to confirm it, a further series of experiments has been carried out, in which rabbits have been submitted for longer or shorter periods to an atmosphere deficient in oxygen.

---

14. In the obtaining of electrocardiograms and their interpretation we were aided by Dr. Harold D. Levine, of Boston.

SECOND SERIES: CARDITIS RESULTING FROM EXPERIMENTAL
LOWERING OF ATMOSPHERIC OXYGEN

Method and Material.—By combining a bell jar with a spirometer an apparatus (figure) has been constructed in which rabbits can be placed in an atmosphere abnormally high in nitrogen and low in oxygen. The expired carbon dioxide and water vapor are absorbed by soda lime through which the gases circulate. Eighteen experiments were performed. In each of these an animal was inoculated intratesticularly with virus III and immediately thereafter placed in the bell jar, and the jar was flushed with nitrogen several times. The system was then closed, but at five minute intervals samples of gas were withdrawn and the concentrations of oxygen, carbon dioxide and nitrogen determined. The maximum and minimum readings of oxygen content were 12 and 4.5 per cent in the several experiments, and in each experiment the oxygen content decreased progressively from the beginning to the end of the period. When the concentration of oxygen approached 4.5 per cent, the animal became unconscious and had to be removed from the chamber. There was considerable variation among animals in the degree of oxygen deprivation that caused collapse. The time during which exposure occurred was determined by the tolerance of the animal to the anoxia and by the amount of oxygen in the chamber at the beginning of the experiment. In the 18 experiments the time of exposure ranged between six and forty-one minutes, but

TABLE 3.—*The Incidence of Cardiac Lesions in Rabbits Subjected to an Atmosphere Deficient in Oxygen*

Animals Treated	Animals with Cardiac Lesions Caused by Virus	
	Number	Percentage
18..	12	66.7

in the majority it was twenty to thirty minutes. As in the preceding group of experiments, the rabbits were killed three to six days after inoculation and exposure to oxygen-deficient atmosphere. The hearts, the testes and the brains were examined for inflammation and inclusion-bearing cells.

Results.—The hearts of 12 of the 18 animals inoculated with virus III and thereafter deprived of the normal amount of atmospheric oxygen contained inflammatory lesions in which there were virus III inclusion bodies (table 3). These lesions were predominantly in the heart muscle (myocarditis). but were occasionally found in the endocardium or the epicardium. Although their incidence, 66.7 per cent, was comparable to that in the previous group of experiments in which acacia, epinephrine, barium chloride or betahypophamine ("pitressin") was used as the localizing factor the individual lesions tended to be less severe. There were no lesions in the brain.

SUMMARY AND CONCLUSIONS

It has been shown that inflammatory lesions occur on occasion in the muscle and membranes of the heart in rabbits suffering from a variety of viral infections. However, the incidence and the severity of

these lesions are markedly increased when the animals are submitted to procedures that tend to decrease the amount of oxygen supplied to the heart. The procedures that have been successful in inducing virus to localize in the heart and there cause inflammation are (1) the administration of drugs that diminish the coronary blood flow both by active constriction of the arteries—betahypophamine ("pitressin"), barium chloride, epinephrine—and by passive compression due to the contracting myocardium and (2) the administration of substances that interfere with the passing of oxygen from erythrocyte to tissue (acacia).

Finally, the fact that anoxia is the determining factor in the occurrence of this experimental viral carditis has been demonstrated in direct experiments in which anoxia was produced by placing the experimental animal in an atmosphere deficient in oxygen.

SIMULTANEOUS CHROMAFFIN TUMORS OF THE CAROTID BODY AND THE GLOMUS JUGULARIS

G. F. KIPKIE, M.D.[*]

MONTREAL, CANADA

S INCE the recognition of the right and left carotid bodies, there have been numerous isolated reports of tumors arising from them. The first was published by Marchand in 1891. In 1929 Bevan and McCarthy[1] collected 133 cases from the literature and added 1 of their own. In 1931 Rankin and Wellbrock[2] added 12 cases, including a case of bilateral tumor. Chase[3] in 1933 reported 2 cases from this institute. The patients were sisters, and in one of them the tumor was bilateral.

Tumor of a carotid body associated with tumor of other chromaffin tissue is rare. Gilford and Davis[4] described a carotid body tumor with a nodule in the liver resembling the tumor of the carotid body. In 1934 Clagg[5] reported tumors occurring simultaneously in a carotid body and an organ of Zuckerkandl. Goodof and Lischer[6] in 1943 recorded a carotid body tumor with a histologically similar tumor of the body of the pancreas. More recently, Rosenwasser[7] described a carotid body tumor arising in the left middle ear and mastoid process and suggested as its origin the glomus jugularis. This structure is described as being found in the apex of the bulb of the jugular vein, immediately below the bony floor of the middle ear and near the ramus tympanicus of the glosso-pharyngeal nerve.[8] In view of all these reports the description of another single case of carotid body tumor would seem to be redundant. However, in the case to be reported there were two chromaffin tumors, one in the right carotid body area and one in the petrous portion of the left temporal bone. Since it is the second reported case of a carotid body-like tumor arising in the temporal bone, the rarity of this site of origin and the duality make it a case of unusual interest.

---

1. Bevan, A. D., and McCarthy, E. P.: Surg., Gynec. & Obst. 49:764, 1929.
2. Rankin, F. W., and Wellbrock, W. L. A.: Ann. Surg. 93:801, 1931.
3. Chase, W. H.: J. Path. & Bact. 36:1, 1933.
4. Gilford, H., and Davis, K. L. H.: Practitioner 18:729, 1904.
5. Clagg, R. W.: Arch. Path. 18:635, 1934.
6. Goodof, I. I., and Lischer, C. E.: Arch. Path. 35:906, 1943.
7. Rosenwasser, H.: Arch. Otolaryng. 41:64, 1945.
8. Guild, S. R.: Anat. Rec. (supp. 2) 79:28, 1941.

REPORT OF A CASE

A Jewish woman was first admitted to the Royal Victoria Hospital in December 1933. At that time she was 29 years of age. Her complaint concerned a discharge of the left ear which had persisted for one year prior to admission. Her own physician on two occasions had removed polyps from the left external auditory canal, but polypoid growth had recurred. When she was examined, there was no tenderness over the mastoid process, but the external auditory canal was blocked in its inner portion by a granular growth about which there was some bloody discharge. A first stage radical left mastoidectomy was performed. The polypoid growth of the external auditory canal was found to extend into the cells of the mastoid process a short distance from the middle ear. Pathologic examination of tissue fragments from the external ear revealed an appearance suggestive of angiomatous polyp. The second stage of the operation was completed nine days later and was followed by an uneventful recovery.

The patient entered another hospital on March 29, 1943. At this time she stated that in 1938 she had first noticed swelling in the right side of the neck and that this swelling had slowly enlarged. A purulent discharge of the left ear had been present since December 1942. On March 31 a biopsy specimen was removed from a polyp discovered in the left auditory meatus, and an attempt was made to remove the cervical tumor. There was severe hemorrhage before the completion of the latter operation, so that the tumor was not removed. About eight hours after operation the patient had palsy of the left side of the· face, some difficulty in speaking and paralysis of the left arm and leg. On April 1 the right pupil was dilated and fixed and the left pupil small and fixed. A lumbar puncture was done several hours later, and a diagnosis of hemorrhage of the right internal capsule was made. The neoplasm in the right side of the neck was believed to be a tumor of the carotid body.

The patient was transferred on April 1 to the Montreal Neurological Institute for emergency operation. She was unconscious and on admission was taken to the operating room for ventriculography. She died before this was completed. The impression was that there was at least one and perhaps more expanding lesions in the right cerebral hemisphere. Roentgen examination disclosed that a considerable portion of the apex of the petrous portion of the left temporal bone was destroyed, probably by neoplasm.

Postmortem Examination.—A complete autopsy was performed four hours after death by Dr. H. B. Jackson. The pertinent findings were confined to the head and the neck, and only these will be described in the following summary.

A mass, which measured 2.5 cm. in diameter and extended upward for a distance of 6.5 cm., was found at the bifurcation of the right common carotid artery. It appeared to have a well defined capsule. A catgut suture completely surrounded the external carotid artery 3 cm. above the bifurcation. One centimeter above the origin of the internal carotid artery a catgut suture was found passing through the lumen of the vessel, occluding about half of its circumference. Jelly-like blood clot was found above the ligature. At the upper pole of the tumor there was a lymph node measuring 1.5 cm. in diameter.

The pyramid of the petrous portion of the left temporal bone was destroyed by a soft tissue mass, but this had not penetrated the dura. The neoplasm extended into the middle ear cavity and into the left external auditory canal. It was soft and lobulated, having the same consistency as pancreatic tissue.

In the brain, the gray and the white matter and the basal ganglions on the right side presented extensive recent softening.
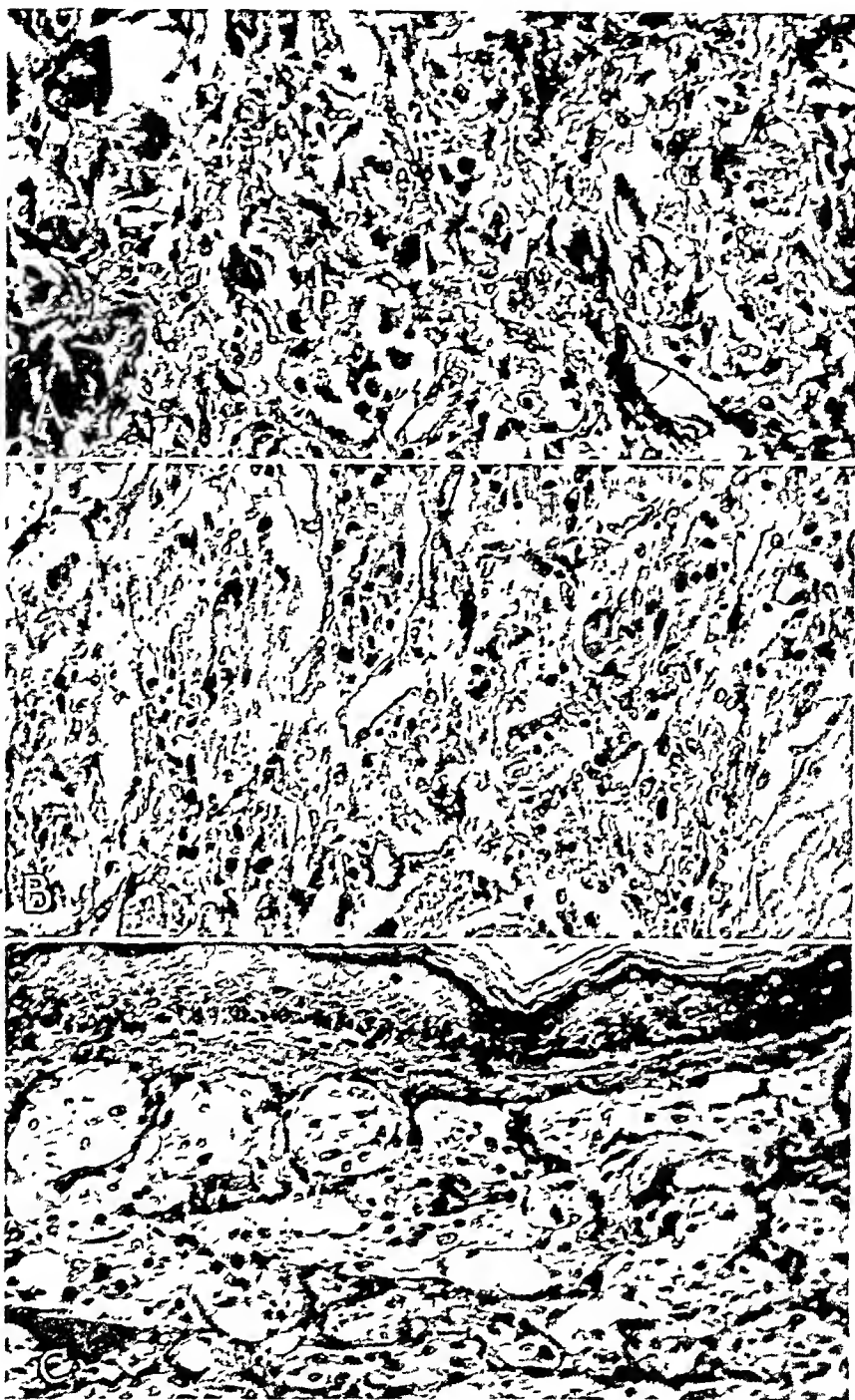
A, photomicrograph of tumor of the right carotid body. The vacuolated cells present possessed finely granular eosinophilic cytoplasm. Hematoxylin-eosin stain; × 230.

B, photomicrograph of tumor of the petrous portion of the left temporal bone. The cytoplasm of the clear cells which encroach on the bony trabeculae was eosinophilic and finely granular. Hematoxylin-eosin stain; × 230.

C, photomicrograph of the original biopsy specimen from the left external auditory canal. Stratified squamous epithelium of the external auditory canal overlies nests of tumor cells immediately beneath. Hematoxylin-eosin stain; × 200.

Microscopic Examination.—After suitable fixation, blocks were cut from the tumor removed from the right side of the neck and the one from the left petrous temporal bone and stained with hematoxylin and eosin, Masson's trichrome stain, Schmorl's stain for chromaffin granules, Verhoeff's stain for elastic tissue and reticulum stains. The cervical tumor was well encapsulated and at the periphery an occasional arteriole contained recent thrombotic material. It was composed of nests and sheets of cells separated by bands of collagenous fibrous connective tissue. In most instances the nests contained six to eight cells, which tended to have a pseudoalveolar arrangement with small capillaries and sinusoids at their peripheries. A delicate fibrous network was scattered throughout the tumor. The cells of the tumor were irregularly polyhedral, although some appeared to have fine fibrils extending from them. The cytoplasm was finely granular and eosinophilic with hematoxylin and eosin. A few of the cells possessed clear, almost colorless cytoplasm. The nuclei were spherical. Many were solid and basophilic, while others were vesicular, with one to three nucleoli. An occasional cell contained two or three hyperchromatic nuclei, but no mitotic figures were discerned. With Schmorl's chromaffin stain, some of the cells were seen to contain bluish green granules, although many were clear (*A* in figure). No tumor cells were found in the lymph node removed from the upper pole of the tumor.

The same histologic picture was encountered in the tumor removed from the petrous portion of the left temporal bone, although here the general tissue structure was looser (*B* in figure). Here, too, Schmorl's stain showed some of the tumor cells to be filled with bluish green granules. In this tumor there were moderate numbers of spicules of bone. Many of these were completely viable, but others were basophilic in their staining capacities. Some had tumor cells in close contact with them, while in other areas multinucleated cells possessing four to five pale basophilic nuclei and eosinophilic cytoplasm were encountered about the necrotic bony spicules.

When the biopsy specimens taken from the left external auditory meatus in December 1933 were reviewed, two histologic pictures were apparent. The tissue fragments were covered by unremarkable stratified squamous epithelium on which there was a little lamellated keratin. Beneath this epithelium, granulation tissue containing numerous blood capillaries and supported by older, partially hyalinized fibrous connective tissue was seen. However, at the base of one of the tissue fragments a different histologic picture was encountered. Here there were a few small nests of four to eight clear cells clumped together and separated by delicate strands of fibrous connective tissue. These cells had the same histologic appearance as those in the tumors of the neck and the petrous portion of the temporal bone. Small capillaries were present at the edges of the nests. Some of the cells had pale granular eosinophilic cytoplasm when stained with hematoxylin and eosin. Their nuclei were spherical or oval. Some were solid and hyperchromatic, while many were vesicular, with nucleoli. An occasional cell contained two or three nuclei, but no mitotic figures were seen (*C* in figure). Schmorl's stain for chromaffin granules revealed a rare cell containing bluish green granules.

COMMENT

Thus, in brief, there are, in this case, a tumor of the right carotid gland and a tumor of the petrous portion of the left temporal bone. The latter would appear to have been present for at least ten years, having been incompletely removed at a previous mastoidectomy.

In view of the rarity of dual carotid body–like tumors the question is raised as to whether this was a cancer of the carotid body of the right side of the neck with metastases in the petrous portion of the left temporal bone. The histologic picture of the tumor of the carotid body was not that of a cancerous lesion. It was encapsulated, there was no invasion of the capsule and no mitotic figures were seen. Moreover, the cervical tumor would appear to have arisen five years after the discovery of a tumor of identical structure in the petrous portion of the left temporal bone and external ear. The tumor of the petrous portion of the left temporal bone, although locally invasive, destroying bone in the immediate vicinity and extending into the middle and external ears, did not appear cancerous histologically. Also, the route by which tumor cells could have reached the opposite side of the neck to produce a metastasis in the right carotid body is not clear.

It would seem more reasonable to assume that there are here two independent tumors of the chromaffin system, one arising in the right carotid body and the second in a more obscure chromaffin structure. The discovery by Guild [8] of a chromaffin structure in the temporal bone, the glomus jugularis, provides a morphologic explanation of the origin of a tumor of this type in the petrous portion of the temporal bone.

The chromaffin tumor of the petrous portion of the left temporal bone is similar in many respects to the one recently reported by Rosenwasser. Both patients when originally presenting themselves complained of a growth in the external canal of the left ear and diminishing hearing. Rosenwasser's patient disclaimed discharge of the ear until four weeks prior to admission to the hospital for mastoidectomy, while the patient in the present case had aural discharge intermittently for eleven years prior to death. The growth in the left external auditory canal in both cases had apparently been present for ten years. The original biopsy specimen from the external auditory canal was reported as "vascular granulation tissue" in Rosenwasser's case and as "angiomatous polyp" in the one presented here.

At mastoidectomy both the tumors resembled granulation tissue and bled excessively. Microscopically, the histologic picture of the tissue removed at mastoidectomy in Rosenwasser's case and the appearance of that in the base of the original biopsy specimen from the external auditory canal in 1933 in our case and of the tissue found in the petrous portion of the left temporal bone at autopsy were essentially similar. Furthermore, the same picture was thought to be present in Rosenwasser's case when the original biopsy specimen from the external auditory canal was reviewed. The tumor nests of relatively large cells in all were separated by relatively dense fibrous connective tissue and tended to be bordered by capillaries and sinusoids. In some areas the cells formed sheets and cordlike structures. Some of the cells of both cases contained

vacuolated cytoplasm, and no mitotic figures were found in any of the sections. Occasional cells in the case reported here had bluish green granules in them, although none were demonstrated in that of Rosenwasser.

SUMMARY

A case of a tumor of the carotid body of the right side of the neck associated with a histologically similar tumor of the petrous portion of the left temporal bone is presented. The case is interesting because of the rarity of multiple tumors arising in the chromaffin system and because of the possibility that the tumor of the petrous portion of the tempporal bone arose from a small and only recently recognized body known as the glomus jugularis. It is also noteworthy that the tumor of the petrous portion of the left temporal bone would appear to have been present for five years before the tumor of the right side of the neck was discovered and for ten years prior to death.

PATHOGENESIS OF GLOMERULONEPHRITIS AND RHEUMATIC FEVER ·

In Vivo Activation of Tissue Antigens as a Result of Streptococcic Infection and Consecutive Formation of Autoantibodies

PHILIP A. CAVELTI, M.D.

SAN FRANCISCO

IN PRECEDING publications it has been shown that autoantibodies to kidney,[1] heart and connective tissue[2] can be produced in rats and rabbits by immunizing the animals with emulsions of the homologous tissues in conjunction with killed streptococci. It has further been reported that in rats such autoantibodies can be pathogenic in that they react with the tissues in situ, autoantibodies to kidney thus producing glomerulonephritis[3] and autoantibodies to heart and connective tissue producing cardiac lesions.[4] It has been pointed out that these results lend support to the view that autoantibodies to certain tissue substances might play a role in the genesis of human glomerulonephritis and rheumatic fever. The first step of this pathogenetic mechanism—during the streptococcic infection preceding these diseases—would consist of the formation of an autogenous antigen, which is effected when streptococci or their products come in contact with substances of certain tissues of the host, such as kidney, heart or connective tissue, the streptococcic and tissue material combining in such a way that the product acts as an antigen. In response to this autoantigen, autoantibodies would be formed, and these autoantibodies in turn would react with the corresponding tissues in situ, thus producing glomerulonephritis or rheumatic fever.

In the experiments described so far,[5] the streptococcus-tissue antigens—corresponding to the hypothetic autoantigen in man—were obtained in vitro by mixing killed streptococci with emulsions of the tissues. The question arose whether such autogenous tissue antigens can

1. Cavelti, P. A., and Cavelti, E. S.: Arch. Path. **39**:148, 1945; **40**:158, 1945.

2. Cavelti, P. A.: Arch. Path., to be published.

3. Cavelti, P. A., and Cavelti, E. S.: Arch. Path. **40**:163, 1945.

4. Cavelti, P. A.: Arch. Path., to be published.

5. Footnotes 1 to 4.

also arise in animals through the effect of streptococcic substances on the tissues in vivo. It is the purpose of the present paper to describe the experiments carried out with regard to this problem.

The general procedure of these experiments was to establish focal streptococcic infections or, in some cases, to inject large amounts of killed streptococci into rats and to investigate by serologic tests (*a*) whether a tissue antigen could be demonstrated in the serum of these rats by the use of a potent antiserum to rat tissues and (*b*) whether subsequently autoantibodies were formed, demonstrable by the use of rat tissue extracts as test antigens.

MATERIALS AND METHODS

Rats of the Evans strain were used throughout (rats of the Curtis-Dunning strain were not available at the time of these studies).

The streptococci used most extensively were strain Dochez N. Y. 5 and its animal passage product E14. Two other stains of group A streptococci, derived from local human sources (nos. 17 and G22), were also employed in some of the rats.

The organisms were administered in the following preparations:

1. The bacterial sediment of 5 cc. of a twenty-four hour culture in beef heart broth was resuspended in a small amount of distilled water and mixed with 3 parts of warm agar. While still fluid, 1 cc. of the mixture was injected subcutaneously into each rat.

2. The organisms were grown for twenty-four hours on beef heart broth containing ground meat (beef heart). Meat pieces 3 to 6 mm. in diameter were implanted subcutaneously in the region of the chest under aseptic conditions, the incision being closed by suture and sealed with paraffin.

3. The organisms were grown on blood agar plates for twenty-four hours. Pieces of agar about 6 mm. in diameter were then similarly implanted under the skin.

4. The organisms were grown for twenty-four hours on a pure soybean broth in the presence of chopped pieces of soybeans. A piece of soybean was then implanted subcutaneously.

5. The organisms were grown for twenty-four hours on a medium consisting of 1 part defibrinated rat blood and 2 parts isotonic solution of sodium chloride and 1 per cent dextrose. From 0.5 to 1 cc. of this culture was injected subcutaneously.

6. Organisms were grown on Bernheimer's synthetic medium [6] and lyophil-dried in the living state. These organisms were mixed with mineral oil and injected subcutaneously in quantities of 2 to 3 mg. of dried streptococci per rat.

7. Organisms grown on Bernheimer's synthetic medium were precipitated (killed) with cold acetone, washed repeatedly with acetone and dried. They were resuspended in saline solution and injected subcutaneously and intraperitoneally in quantities of 40 to 100 mg. of dry organisms per rat.

These materials were administered in one injection or one implantation.

6. Bernheimer, A. W.; Gillman, W.; Hottle, G. A., and Pappenheimer, A. M., Jr.: J. Bact. **43**:495, 1942.

Immediately before each injection or implantation and at various intervals thereafter the rats were bled from the tail (0.5 to 1 cc. of blood being withdrawn).

Rat kidney, heart and connective tissue perfused free of blood were obtained, and 20 per cent emulsions of these tissues were prepared and preserved as described previously.[5] For serologic tests extracts of the frozen emulsions were made by centrifugation. The collodion particle technic was used exclusively for the in vitro tests as described in detail elsewhere.[7]

Antiserums to rat kidney, heart and connective tissue were prepared by immunizing rabbits with the tissue emulsions. A total of 20 or more intraperitoneal injections were given to each rabbit at intervals of three to five days, the single dose being 5 cc. of the 20 per cent emulsions or the equivalents of emulsions made from lyophil-dried tissues. Blood was obtained from the rabbits on the seventh day after the last injection. The serum was filtered through a Seitz filter and merthiolate was added, 1 : 10,000, for preservation.

RESULTS

In most of the rats the site of the focal infection showed marked inflammatory swelling during the first few days, which then gradually diminished and disappeared within seven to fourteen days. In a few rats an abscess perforated through the skin and discharged.

Serum samples taken from the rats twelve and thirty-six hours (or twenty-four and forty-eight hours in other experiments) after the initiation of the focal infection were tested for the presence of autogenous tissue antigens with serums from rabbits immunized against rat kidney, heart and connective tissue. Of each of the several serums from rabbits immunized against the same tissue, the one showing the strongest in vitro reaction was chosen. By far the most potent' was the antikidney serum, which gave a 4 plus reaction in a dilution of antigen of at least 1 : 320 (the extract of 20 per cent emulsion of kidney taken as undiluted), while the antiserum to rat heart and that to connective tissue showed 2 plus reactions with antigen dilutions of 1 : 80 and 1 : 40, respectively. When the titers of these antiserums were expressed in terms of serum dilution, the differences did not emerge so clearly, but for the purpose of determining an antigen as intended the former point of view obviously is more pertinent.

The organ or tissue specificity of these antiserums was not very high. The antiheart and anti-connective-tissue serums showed extensive cross reactions with connective tissue and extract of heart, but they did not react at all or only slightly with extract of rat lung, liver or kidney. Attempts to differentiate between antibodies to rat heart and those to rat connective tissue by means of absorption with either one of these tissues did not succeed. The antikidney serum reacted slightly with rat liver, connective tissue and lung, somewhat more strongly with rat skeletal muscle and strongly with rat heart. When absorbed with sedi-

---

7. Cavelti, P. A.: (*a*) J. Immunol. **49**:365, 1944; (*b*) to be published.

mented particles from rat heart emulsion, all reactivity except that with kidney was removed, but the reaction with kidney was appreciably impaired. None of these antiserums showed any reaction whatsoever with normal rat serum under the conditions of the test, nor did they react with suspensions or extract of streptococci or culture filtrate or beef heart broth.

In the tests for the presence of tissue antigens in rat serum during focal streptococcic infection, the rabbit anti-rat-heart and anti-rat-connective-tissue serums were used unabsorbed, and the anti-rat-kidney serum was employed unabsorbed and also after it had been absorbed with rat heart. In small tubes with a diameter of 7 mm., 0.1 cc. of a 1 :10 dilution of stock collodion particle suspension was added to 0.1 cc. of undiluted rat serum, quickly followed by 0.1 cc. of the undiluted antiserum. After mixing, the tubes were incubated at room temperature for one hour, then centrifuged for three minutes at 1,400 revolutions per minute, and the results were read. After keeping the tubes in the refrigerator for six hours and recentrifuging, the results were read again.

Reactions were obtained only in serum samples taken between twelve and forty-eight hours after initiation of the infection, the maximum reaction occurring after twenty-four to thirty-six hours. Reactions considered significant (2 plus or stronger) were recorded for 21 of the total of 46 rats. Most of these reactions occurred with the antiserum to rat kidney when the latter was used unabsorbed. With the anti-rat-kidney serum that had been absorbed with rat heart and with the anti-rat-heart and anti-rat-connective-tissue serums only a few weak reactions were observed.

The results obtained indicate that during focal streptococcic infection, in these rats, one or several tissue antigens were released into the blood stream. It is, however, not possible to decide from these results what tissues yielded these substances.

Serum samples obtained from the same rats at intervals later on were tested for the presence of autoantibodies, with extract of rat kidney and rat heart as test antigens in the same manner as described in detail elsewhere.[8] To 0.5 cc. of each of progressive serum dilutions 0.2 cc. of collodion-antigen mixture was added, and after the tubes had been incubated for one to two hours at room temperature and then centrifugated at 1,400 revolutions per minute for three minutes the tests were read. Autoantibodies could be demonstrated during a period of between eight and fourteen days after the onset of the infection. Autoantibodies to rat heart were demonstrated in the serums of 18 of the 46 rats (barring reactions in serum dilutions up to 1 :20, the specificity of which is questionable). There was no marked difference in effectiveness between the various mediums and vehicles used for establishment of the focal infec-

---

8. Footnotes 1, 2 and 7.

tions. However, the incidence of reactions was much lower in serums of the rats given injections of killed (acetone-precipitated) organisms, even when large doses were employed.

Autoantibodies giving definite in vitro reactions with extract of rat kidney were demonstrated in the serums of 6 rats. These serums failed to react with extract of rat heart.

With the use of two pools, each composed of 6 serums reacting with extract of rat heart, about equally strong reactions were obtained with extracts of heart, skeletal muscle and connective tissue, while practically no reaction was observed with extracts of rat lung, liver, brain or kidney.

It must be pointed out that, on the whole, all of these reactions were much weaker than those obtained after rats had been immunized with mixtures of streptococci and rat tissues (heart, connective tissue, etc.), which have been reported elsewhere.[2]

Histologically, significant cardiac or renal lesions occurring as a result of a pathogenic effect of the autoantibodies formed were not observed. However, 2 of the rats showing autoantibodies to kidney did exhibit slight urinary changes consisting chiefly of mild proteinuria and some microscopic hematuria; in 1 of these the changes persisted for a few months but finally subsided.

COMMENT

According to the results reported, it appears that in rats focally infected with group A beta hemolytic streptococci autogenous tissue antigens are formed in consequence of some effect of the streptococci or their toxins and are released into the blood stream. These tissue antigens can be demonstrated by means of potent antiserums to rat tissues produced in the rabbit. Further, as a response to these autogenous antigens, autoantibodies are formed, demonstrable by the use of extracts of rat tissues as test antigens.

In the studies reported it was not possible to decide from what organs or tissues these autoantigens originated. The antikidney serum by means of which the most definite in vitro reactions were obtained reacted strongly not only with rat kidney but also with rat heart. After this serum had been absorbed with rat heart, its reactivity was limited to kidney, but the autogenous antigen in rat serum could not be definitely demonstrated with this absorbed serum. This apparent removal of the antibodies to the antigen in question by heart absorption of the antikidney serum seems to indicate that this antigen is one which is present not only in kidney but also in other tissue such as heart. The fact that the rabbit anti-rat-heart serum failed to yield definite reactions with the autogenous antigen does not necessarily conflict with this view, as this failure may be due to the fact that this serum was markedly weaker than the antikidney serum.

The results obtained with the serum samples containing autoantibodies to the autogenous antigen also support the view that the latter was derived, at least in most instances, from the heart or other tissues containing the same antigen, rather than from the kidney, in that the majority of the reactive serums showed reactions with extracts of heart rather than kidney. It should, however, be mentioned again that some of the serums reacted with kidney and not with heart and that the anti-kidney serum that had been absorbed with rat heart gave slight reactions with a few of the rat serums containing autogenous antigens. In these cases it may be that the latter originated from the kidney.

When the results of the tests for autogenous antigens and autoantibodies were compared with respect to each individual rat, no very definite correlation was evident; i. e., rats whose serum showed relatively strong reactions in tests for autogenous antigen did not always develop autoantibodies in demonstrable amounts and vice versa. But in view of the relatively weak reactivity of the serums on the whole, this inconsistency is not surprising.

Although the evidence presented for the phenomenon described is not very strong, especially in the sense that the intensity of the serologic reactions obtained did not far exceed the minimum detectable by the method employed, it should be pointed out that these experiments were carried out in three different sets at different times and that each time essentially the same results were obtained.

The results of the experiments reported lend further support to the view that autoantibodies to certain tissues may play a role in the genesis of human glomerulonephritis and rheumatic fever. They seem to indicate in principle the existence of the possibility that during the streptococcic infection preceding these diseases autogenous tissue antigens are activated by the effect of streptococcic substances on the tissues of the host. Experimental evidence that, as a response to antigenic tissue-streptococcus combinations, autoantibodies are formed and, owing to the pathogenic effect of the latter, renal and cardiac lesions develop, has already been presented in preceding papers.

The phenomenon described here may have some resemblance to the findings in rheumatic fever, reported by Coburn and Pauli,[9] and further studied by Wedum and Wedum,[10] according to which an antigen was present in the serum of rheumatic subjects during the prerheumatic phase, the antibody to which appeared at the onset of the rheumatic attack. One difference, however, is that in the present experiments the autogenous antigen appeared during the height of the streptococcic infection, while the antigen reported by Coburn was present at a time two to

9. Coburn, A. F., and Pauli, R. H.: J. Exper. Med. **69**:143, 1939.

10. Wedum, A. G., and Wedum, B. G.: Proc. Soc. Exper. Biol. & Med. **61**: 432, 1946.

three weeks after the onset of the streptococcic infection, i. e., in most instances after subsidence of the acute infection and preceding the onset of the rheumatic attack by only a few days.

SUMMARY

In rats focally infected with group A beta hemolytic streptococci, autogenous tissue antigens appear to be released into the blood stream during the height of the infection. These antigens were demonstrated by means of antiserums to rat tissues produced in the rabbit.

As a response to these autogenous antigens, autoantibodies are formed, as demonstrated by the use of extracts of rat tissues as test antigens.

CORRELATIONS OF BIOCHEMICAL AND HISTOLOGIC CHANGES IN THE ADRENAL CORTEX

WALTER F. ROGERS Jr., M.D.

AND

ROBERT H. WILLIAMS, M.D.

BOSTON

THE IMPORTANT functions of the cortex of the adrenal gland have made it the subject of increasingly extensive investigation. As the individual hormones of the cortex have been isolated and their physical and chemical characteristics established, attempts have been made to use histochemical technics to demonstrate their sites of production and storage, and to relate these observations to the activity of the various zones of the cortex. However, the specificity of the histochemical methods used and their correlation with the biochemical changes occurring in this gland have not heretofore been adequately evaluated.

The histologic methods that have been proposed for the detection of biologically active steroids include staining with phenylhydrazine,[1] use of Schiff's reagent,[2] digitonin precipitation and use of reagents of the Leibermann-Burchard test.[3] Estimations have also been made of the amount of birefringence and autofluorescence of the adrenal cortex as examined by polarized light and ultraviolet rays, respectively,[4] and sudan dyes have been used in the estimation of the lipoid content of the adrenal glands for many years.

In 1943 Sayers and associates[5] reported a decrease of adrenal cholesterol following administration of an adrenotropic extract of the pituitary gland. This interesting observation facilitated the interpretation of the previously known fact that the cholesterol content and the

1. Bennett, H. S.: (a) Proc. Soc. Exper. Biol. & Med. **42**:786, 1939; (b) Am. J. Anat. **67**:151, 1940.

2. Feulgen, R., and Voit, K.: Arch. f. d. ges. Physiol. **206**:389, 1924.

3. (a) Dempsey, E. W., and Bassett, D. L.: Endocrinology **33**:384, 1943. (b) Dempsey, E. W., and Wislocki, G. B.: ibid. **35**:409, 1944.

4. Deane, H. W., and Greep, R. O.: Am. J. Anat. **79**:117, 1946.

5. Sayers, G.; Sayers, M. A.; White, A., and Long, C. N. H.: Proc. Soc. Exper. Biol. & Med. **52**:200, 1943.

sudanophilic material of human adrenal glands vary greatly under different circumstances,[6] in general being low in patients with severe infections, moderately decreased in those with chronic debilitating diseases and cancer and high in people who die of cardiorenal disorders.

During the last two years we have determined the cholesterol content of the adrenal glands in selected cases coming to autopsy and have studied these glands with some of the previously mentioned histochemical technics. In this paper, the observations made histologically are compared with the cholesterol contents of the adrenal glands, and an attempt is made to evaluate the significance of the findings.

MATERIAL AND METHODS

The adrenal glands used in this study were from the Mallory Institute of Pathology; some of the glands were supplied by Dr. T. Leary, medical examiner for Suffolk County, Southern District. The adrenal glands were removed intact from the body. The surrounding fat was carefully trimmed off, and the glands were weighed on a torsion balance. Blocks were then cut and placed in a 4 per cent aqueous solution of formaldehyde and in Zenker's solution. The remaining portions of the glands, usually 80 per cent of the total weight, were kept at — 10 C. until the determinations of the total cholesterol were done by the Schoenheimer-Sperry method as modified by Sperry.[7] The tissue fixed in Zenker's solution was stained with phloxine and methylene blue. Three frozen sections, each 12.5 to 15 microns thick, were cut from the formaldehyde-fixed blocks and were mounted with glycerin jelly, one being unstained, another stained with sudan IV and hematoxylin and the last stained with phenylhydrazine hydrochloride as described by Bennett.[1b] The unstained frozen section was examined by polarized light and ultraviolet rays.

OBSERVATIONS ON NORMAL ADRENAL CORTEX [8]

The microscopic picture of a normal cortex stained with phloxine and methylene blue or hematoxylin and eosin presents three distinct zones, namely, the zona glomerulosa, the zona fasciculata and the zona reticularis (fig. 1 *A*). The outer one or zona glomerulosa is characterized by a moderately thin layer of cells arranged in well circumscribed clusters. The nuclei of these cells stain fairly dark, while the cytoplasm appears

---

6. Wacher, L., and Hueck, W.: Arch. f. exper. Path. u. Pharmakol. **71**:373, 1943. Landau, M., and McNee, J. W.: Beitr. z. path. Anat. u. z. allg. Path. **58**: 667, 1914. Chauffard, A.; Laroche, G., and Grigart, A.: Compt. rend. Soc. de biol. **76**:529, 1914. Kohno, T.: Folia endocrinol. japan. **4**:60, 1928. Ewart, B.: Upsala läkaref. förh. **40**:536, 1934-1935. Sarason, E. L.: Arch. Int. Med. **71**:702, 1943.

7. Sperry, W. M.: Am. J. Clin. Path. (Tech. Supp.) **2**:91, 1938.

8. One must be most careful in the histologic interpretation of normal adrenal glands, for those of vigorous young adults dying of diseases seemingly unrelated to the adrenal cortex will show histologic changes consistent with overactivity of the cortical cells. In this series, normal glands have been considered to be those taken from persons who suffered instantaneous traumatic death and showed at autopsy no lesions other than those caused by the accident.

fluffy and vacuolated. In the outermost portion of the zona glomerulosa, just beneath the capsule, there are frequently smaller cells with dark nuclei and acidophilic cytoplasm. The zona glomerulosa blends into the zona fasciculata, which in a normal adrenal gland is the widest zone and is composed of moderately large polyhedral cells arranged in cords. The nuclei of these cells are larger and lighter staining than those of the glomerulosa. The cytoplasm is fluffy and vacuolated, and these cells have been termed "spongiocytes." The vacuolated appearance of both the zona glomerulosa and the zona fasciculata in sections prepared from paraffin blocks results from the dissolution of the lipoid substances in the process of preparation. The line of demarcation between the zona fasciculata and the inner layer or zona reticularis is not distinct. As the reticular zone is approached there is a gradual transition to cells arranged in an irregular fashion, which have acidophilic cytoplasm and various patterns of nuclei ranging from dark staining or pyknotic nuclei to those with a vesicular character. The cells of this layer lack the vacuolated appearance of those of the other zones and frequently contain a brownish yellow pigment, the function of which is not known.[9]

Birefringence.—As seen in figure 1 *B* and *C,* the birefringence of the normal adrenal cortex is marked. Its concentration is almost entirely limited to the zona glomerulosa and the zona fasciculata, particularly the latter. In tissue fixed in formaldehyde solution, the doubly refractile material is in the form of needle-like crystals, although portions may appear diffusely as fine particles. If the slide is heated for a few seconds over a microscope lamp and then gentle pressure is exerted on the cover slip and the slide allowed to cool, the predominant portion of the birefringent material appears as maltese crosses (fig. 2 *A*), which are characteristic of such substances as cholesterol esters.[10]

Phenylhydrazine and Sudan Stains and Autofluorescence.—Figure 2 *B* shows a normal cortex stained with phenylhydrazine hydrochloride. The cells of the zona glomerulosa and the zona fasciculata, particularly those in the outer portion of the latter, appear light yellow. The zona reticularis is slightly yellow, but the medulla remains unstained. In addition, the fat contained in signet ring cells outside the capsule is also yellow. Figure 2 *C* shows the appearance of the same adrenal tissue when stained with sudan IV.

Under ultraviolet rays the adrenal cortex fluoresces yellowish green, the areas of greatest fluorescence being located in the glomerular and fascicular zones. However, because of the diffusion of the light of the fluorescent material, the effort to localize the material in question exactly to the respective areas is more difficult with this method of study.

9. Blackman, S. S.: Bull. Johns Hopkins Hosp. **68**:180, 1946.

10. White, C. P.: J. Path. & Bact. **13**:3, 1909.

Fig. 1.—*A*, normal adrenal cortex from a 24 year old woman dying instantly of a severed spinal cord. Note the vacuolation and the fluffiness of the cells, particularly of those of the fascicular and glomerular zones. Phloxine–methylene blue; × 127.5. The photomicrographs in figures 1 and 2 are all from sections of the same adrenal gland.

B, normal adrenal cortex examined by polarized light. The birefringence is limited almost entirely to the fascicular and the glomerular zone, particularly to the former.

C, same adrenal cortex as shown in *B* under higher power; × 127.5. The capsule is on the left, while the reticular zone and the medulla are on the right.

If one compares figures 1 *A* and *C* and 2 *B* and *C,* the parallelism of the findings by the various methods is readily appreciated.

OBSERVATIONS ON ADRENAL CORTEX DEPLETED OF LIPOID CONTENT

Whenever the body is subjected to a noxa, whether infection, chemical or physical trauma, roentgen radiation, ·forced muscular exercise, anoxia or extremes of temperature, the adrenal cortex undergoes characteristic changes.[11] These consist of a depletion of the lipoid con-
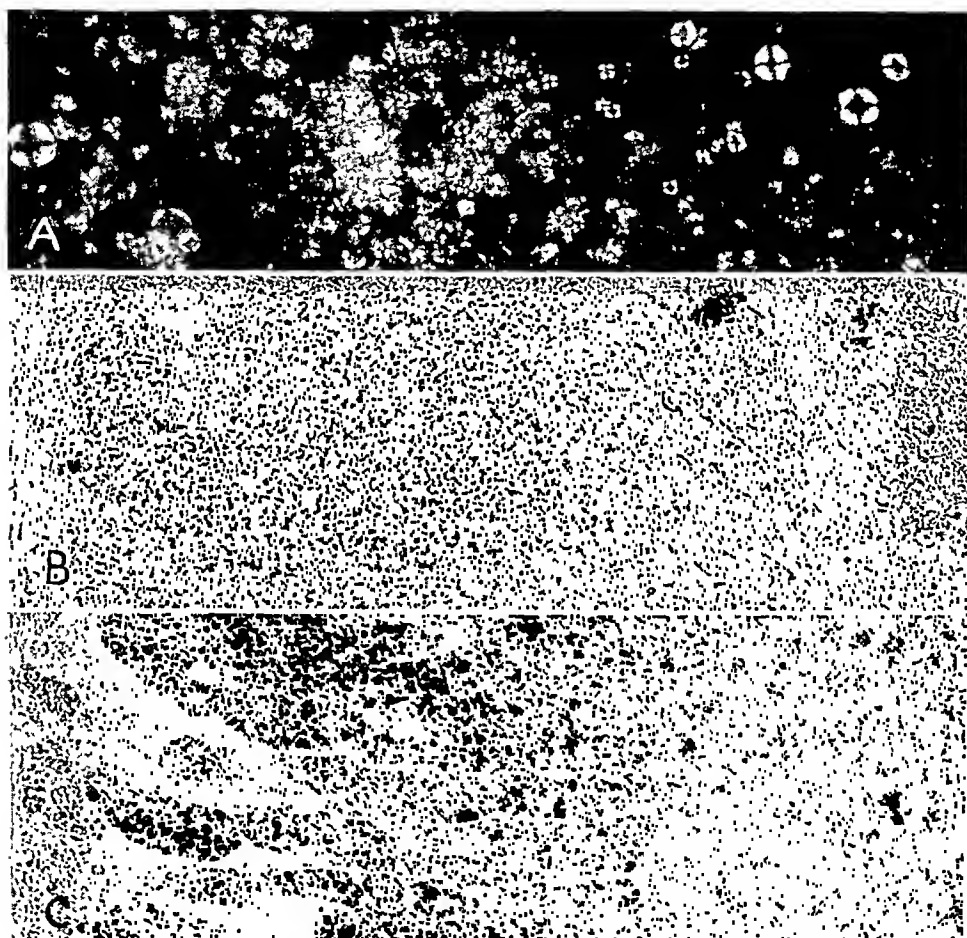

Fig. 2.—*A,* birefringent material of a normal adrenal cortex after application of heat and pressure. Maltese cross formation is characteristic of cholesterol esters. × 127.5.

B, normal adrenal cortex stained with phenylhydrazine. × 127.5. This photomicrograph was made with a blue filter. The darker shade is imparted by the yellow color of the phenylhydrazine. Note that its greatest concentration is in the glomerular zone and the outer part of the fascicular zone. The reticular zone is a lighter yellow, and the medulla remains unstained.

C, normal adrenal cortex stained with sudan IV; × 127.5. Note that the distribution of the stain is similar to that of the birefringence and the phenylhydrazine reaction shown in figures 1 *C* and 2 *B.*

11. Selye, H.: J. Clin. Endocrinol. **6**:117, 1946.

tent, an enlargement and an increase in weight. These phenomena have been interpreted as evidence of increased activity of the cortex, with production of large amounts of corticoid substances, and studies have shown that during periods of "alarm" increased amounts of corticoid substances are excreted in the urine.[12]
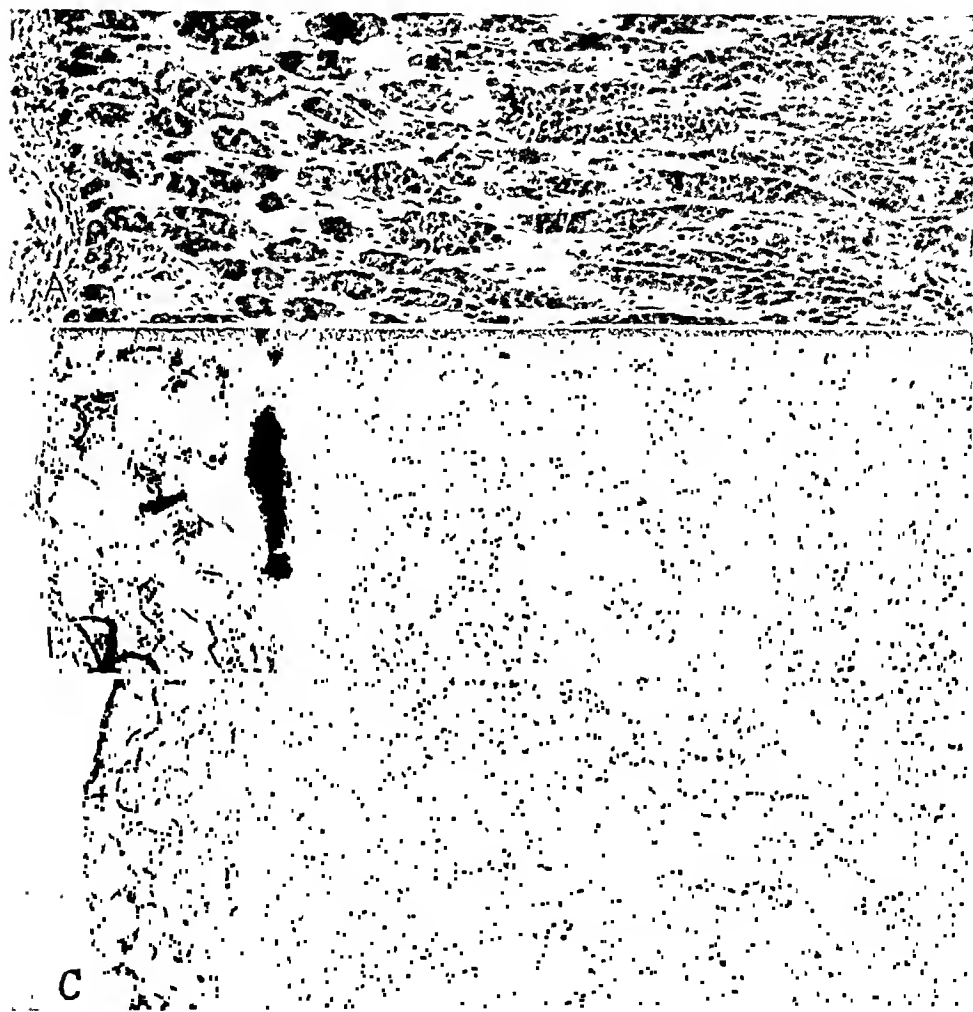

Fig. 3.—*A*, adrenal cortex from a 63 year old man who died of a fulminating streptococcic infection of four days' duration. Phloxine-methylene blue; × 122. Compare with figure 1 *A* and note the absence of vacuolation and the dark staining of the cytoplasm characteristic of an adrenal gland which has been depleted of lipoid material. The photomicrographs shown in *B* and *C* are from this same adrenal cortex.

B, "Lipid-depleted" adrenal cortex stained with sudan IV; × 122. For comparison note the concentration of stainable lipid in fat cells outside the capsule.

C, "Lipid-depleted" adrenal cortex stained with phenylhydrazine; × 122. Note absence of reaction in the cortex and its presence in fat cells. This photomicrograph was made with a blue filter.

12. Weil, P. G., and Browne, J. S. L.: Science **90**:445, 1939. Venning, E. H.; Hoffman, M. M., and Browne, J. S. L.: Endocrinology **35**:49, 1944. Shipley, R. A.; Dorfman, R. I.; Buchwald, E., and Ross, E.: J. Clin. Investigation **25**:673, 1946.

The characteristic picture of the cortex after it has been placed under extreme stress is shown in figure 3. When stained with phloxine and methylene blue, the cells of the zona glomerulosa and the zona fas-
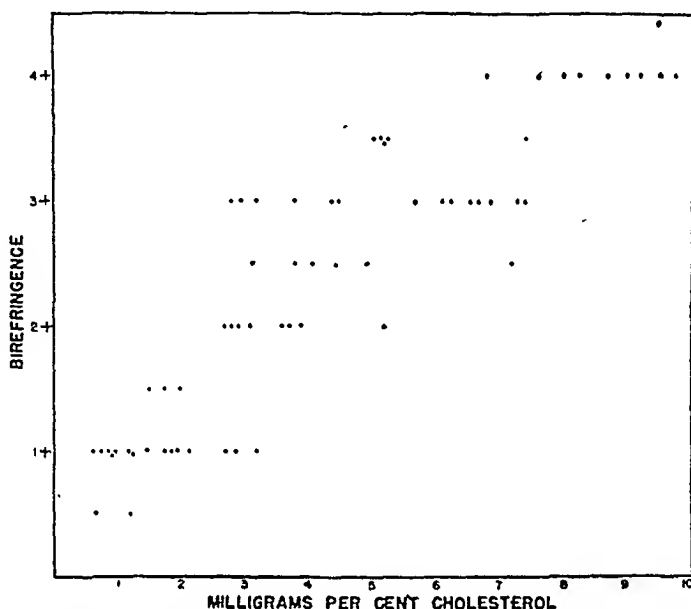
Fig. 4.—The degrees of birefringence plotted against the cholesterol contents of 64 adrenal glands. Note the relationship in this and the two following charts.
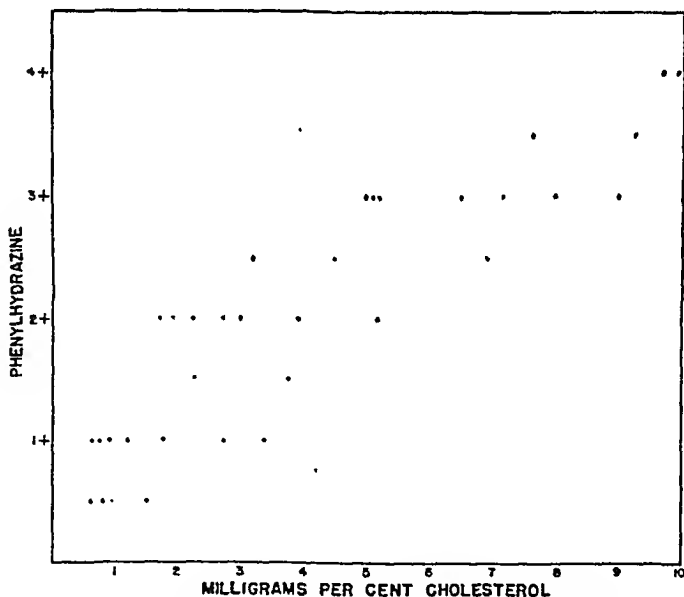
Fig. 5.—The degrees of intensity of phenylhydrazine reaction plotted against the cholesterol contents of 34 adrenal glands.

ciculata are not vacuolated, having lost their lipoid content, and are stained deeply with phloxine (fig. 3 A). The cells appear more compact, while the cords are separated by wider sinusoids and spaces. At times

there is distinct tubular formation among the cells. These changes have frequently been described as resulting from postmortem deterioration, but Dr. N. Zamcheck, of the Army Institute of Pathology, observed in his examination of more than 5,000 adrenal glands that in cases of sudden accidental death the length of time post mortem did not alter significantly the normal histologic appearance. In addition, normal adrenal glands may be left at room temperature for as long as fifty-six hours without development of the changes seen in adrenal glands that have been "depleted" of lipoid substance following great physiologic stress.[13]

When an adrenal gland thus depleted is examined by polarized light, there is no visible birefringent material, and under ultraviolet rays the
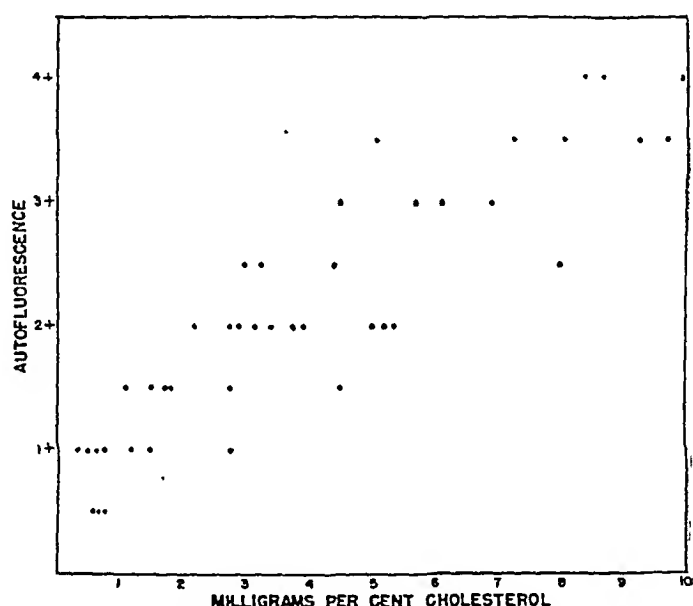
Fig. 6.—The degrees of autofluorescence plotted against the cholesterol contents of 42 adrenal glands.

cortex has a negligible amount of fluorescence. After staining with sudan IV the cortex is completely devoid of sudanophilic material (fig. 3 *B*), and likewise there is no staining with phenylhydrazine hydrochloride (fig. 3 *C*). There are, of course, varying degrees of depletion between the normal cortex and one that has become extensively depleted, but all methods show to an equal degree not only the amount but also the location of the changes.

These results suggest that the methods used merely demonstrate fluctuations in the lipoid content of the adrenal cortex. If this is true, it should be possible to correlate the concentration of total cholesterol

<hr/>

13. Zamcheck, N.: Personal communication to the authors.

with the intensity of the various reactions, as it was shown by Popjak [14] that cholesterol and its esters account for most of the change in the lipoid content of the adrenal gland. Such correlations are shown in figures 4, 5 and 6, in which the amount of birefringence, the intensity of staining with phenylhydrazine hydrochloride and the amount of auto-fluorescence, respectively, are graded from 0 to 4 plus and plotted against the total cholesterol content of the adrenal glands.

In all three figures the correlations are apparent. As the cholesterol content of the gland decreased, so did the intensity of the other reactions. It was also observed, and should be emphasized, that staining with sudan IV is a reliable index to the concentration of cholesterol and the location and the amount of lipoid material.

<div align="center">COMMENT</div>

In the evaluation of these various methods, the principal aspects to be considered are the presumably very small amounts of cortical hormones present in tissue sections, the nonspecificity of the stains used, including the reactions of these stains with cholesterol, and the significance of a decrease of adrenal cholesterol.

If biologically active adrenal hormones are to be demonstrated by histochemical technics, it is well to consider the amount of hormone postulated as present in a tissue section. The average measurements of a normal adult adrenal gland are 50 by 25 by 8 mm., with each gland weighing an average of 4.5 Gm. If a section 15 microns in thickness is cut in the widest portion of an adrenal gland, one can estimate that its average weight would be about 1.35 mg. and its volume 3 cu. mm.

To our knowledge there are no figures concerning the hormonal contents of human adrenal glands; so, for a rough scale of comparison, the values for beef adrenal glands will be used.[15] Olson and associates [16] did extensive studies on the assay of extracts of adrenal cortex and in four potent extracts of beef adrenal glands the average glycogenic potency was equivalent to 8 micrograms of corticosterone per gram of fresh tissue and the renal function potency was equivalent to 4.5 micrograms of desoxycorticosterone acetate per gram of fresh tissue. At 12.5 micrograms per gram of fresh tissue, the amount of hormonally active steroid one would expect to find in an average section of human adrenal gland 15 microns in thickness would be 0.017 microgram. This amount of material would be spread over an area of approximately 200 sq. mm.

14. Popjak, G.: J. Path. & Bact. **56**:485, 1944.

15. When this comparison is made, it is realized that there are distinct species differences in the concentration of cortical hormones per gram of fresh adrenal tissue (e. g., pig as compared with beef).

16. Olson, R. E.; Jacobs, F. A.; Richert, D.; Thayer, S. A.; Hopp, L. J., and Wade, N. J.: Endocrinology **35**:430, 1944.

In comparison, the cholesterol content of a normal human adrenal gland [17] is about 7 mg. per hundred milligrams of tissue, which results in a postulated cholesterol content of 94.5 micrograms per tissue section, or over five thousand times as much cholesterol as estimated active cortical hormone. The probable difficulty of seeing cortical hormones is emphasized by the fact that when the cholesterol content of human adrenal glands drops below 0.5 mg. per hundred milligrams of tissue, it is difficult to see any birefringent material. At this level there is still 6.8 micrograms of cholesterol per tissue section. Accordingly, if one cannot see this concentration of a known amount of birefringent material, it is difficult to conceive how one could detect 0.017 microgram of birefringent material, the postulated concentration of cortical hormone.

Admittedly, chemical extraction is probably inefficient at present, but if cholesterol at a concentration of four hundred times that of cortical hormone cannot be seen, and, by this token, the chemist is extracting only 0.25 of 1 per cent of the active material, the foregoing observations concerning the improbability of seeing cortical hormones would still be valid.

Although the birefringence directly correlates with the cholesterol content of the gland as shown, it is conceivably not due to this constituent alone, particularly in the normal gland. Some of the double refraction may be imparted by phospholipids and other compounds. However, the contribution of such substances to the total birefringence is apparently considerably less than that of cholesterol, as complete extinction of birefringence is noted at low levels of cholesterol despite the fact that the adrenal gland still contains as much phospholipid as it did in the undepleted state.[14] This fact leads one to the conclusion that materials which are birefringent in crystalline form may not be so when in a medium such as a tissue section.

The phenylhydrazine reaction was proposed by Bennett [1b] and has been used by him and other investigators [18] to show the sites of origin and secretion of steroid hormones. However, Gomori [19] pointed out that the phenylhydrazine reaction and Feulgen's leukofuchsin reaction (Schiff's reagent) as used in the so-called "plasmal" reaction [2] had similar sites of localization. Albert and Leblond [20] recently confirmed this. Both groups of investigators showed that the two reactions occurred with many different tissues, particularly with those rich in lipoid material and in such structures as myelin sheaths, tubercles, infarcts, tumors and fat cells themselves. It is the opinion of these

17. Rogers, W. F., Jr., and Williams, R. H.: Unpublished data.

18. Dempsey and Bassett.[3a] Wislocki, G. B., and Bennett, H. S.: Am. J. Anat. **73**:335, 1943.

19. Gomori, G.: Proc. Soc. Exper. Biol. & Med. **51**:133, 1942.

20. Albert, S., and Leblond, C. P.: Endocrinology **39**:386, 1946.

authors that loosely bound aldehydes of high fatty acids are responsible for the reactions. In addition, we have noted in fresh atherosclerotic plaques intense reactions with phenylhydrazine in the same areas which are stained with sudan IV. We have also found that when the cholesterol content of human adrenal glands decreases, predominantly owing to a fall in the cholesterol ester, the intensity of the phenylhydrazine reaction likewise decreases. It thus seems that phenylhydrazine and Schiff's reagent are not reliable for locating ketosteroids, as they both react with certain widespread lipid fractions.

Autofluorescence, reaction with digitonin, and the Liebermann-Burchard test [21] have been suggested as methods of localizing biologically active steroid hormones. However, cholesterol takes part in all of these reactions whether they occur in sections of tissue or in test tubes. For example, cholesterol when viewed by ultraviolet rays fluoresces a light greenish color; its precipitation with digitonin [22] and its reaction with the Liebermann-Burchard reagent [23] in tissue sections are known, and both of these reactions are used in the quantitative determination of cholesterol.[7] Therefore, it seems that these reactions demonstrate chiefly the presence of cholesterol in high concentration rather than active steroid hormones, which are presumably scanty.

When an adrenotropic pituitary extract is administered to rats and guinea pigs, the cholesterol content of their adrenal glands decreases, and, concomitantly, with this change, there is an increase in glycogen in the liver,[24] which is indirect evidence that, accompanying the fall of adrenal cholesterol, there is secretion of adrenal hormones of the corticosterone type. Thus, the cholesterol content of the adrenal gland seems to be of great aid in indicating the physiologic activity of the gland, but until the biochemical mechanism of the production of biologically active steroid hormones and their relationship to cholesterol are established (i. e., until there is a demonstration of cholesterol being transformed into active steroid hormones, or a refutation of the claim that this occurs) the interpretation of the concentration and the location of lipids, particularly cholesterol, must remain limited. This fact, however, does not detract from the value of the investigations which have shown a shift of lipid patterns following such procedures as hypophysectomy and administration of an adrenotropic pituitary extract, biologically active cortical substances and other agents influencing adrenal activity.

21. Dempsey and Bassett.[3a] Dempsey and Wislocki.[3b]

22. Windaus, A.: Ber. d. deutsch. chem. Gesellsch. 42:238, 1909; Ztschr. f. Physiol. 65:110, 1910.

23. Schultz, A.: Zentralbl. f. allg. Path. u. path. Anat. 35:314, 1924-1925.

24. Sayers, G.; Sayers, M. A.; Fry, E. G.; White, A., and Long, C. N. H.: Yale J. Biol. & Med. 16:361, 1944. Sayers, G.; Sayers, M. A.; Liang, T. Y., and Long, C. N. H.: Endocrinology 38:1, 1946.

SUMMARY

A study has been made of the cortices of normal and "lipid-depleted" adrenal glands of man, in which various histologic technics were used, such as staining with phloxine–methylene blue, phenylhydrazine and sudan IV and examination by polarized light and ultra-violet rays.

The changes in the adrenal glands observed with the respective technics were compared with each other and with the quantitative determinations of the cholesterol contents of these glands.

It was found that with all of the technics except the phloxine–methylene blue the changes paralleled one another as to degree and location and that the intensity of reaction was proportional to the cholesterol content.

We have stressed the fact that the histologic methods which have been suggested for the detection of biologically active steroid hormones yield reactions similar to those of cholesterol and that the latter is in high concentrations, while the hormones are presumably in minute amounts.

The methods described can be used to demonstrate the localization and the shift of lipid patterns in the adrenal gland, which in turn give an indication of the amount of activity due to pituitary adrenotropic hormone. However, there is no proof that they indicate the amount or the distribution of active adrenal hormones.

PATHOLOGIC ASPECTS OF LEIOMYOSARCOMA
OF THE STOMACH

CRICHTON McNEIL, M.D.

AND

FRANK E. HESSE, M.D.

SALT LAKE CITY

NEARLY all of the reports concerning leiomyosarcoma of the stomach have dealt with the clinical aspects and the differential diagnosis of sarcoma and carcinoma of the stomach. In this paper the pathologic aspects of leiomyosarcoma of the stomach will be emphasized, and a case will be reported.

REVIEW OF THE LITERATURE

To May 1935 the literature contained reports of 371 cases of sarcoma of the stomach. Three authors, Lubarsch,[1] D'Aunoy[2] and Douglas,[3] reported that 30 to 50 per cent of these cases were cases of either leiomyosarcoma or myosarcoma. Most of the remainder belong in the lymphosarcoma group. In an excellent review of the subject sarcoma of the stomach, Pack and McNeer[4] reported that the tumor occurs on either the greater or the lesser curvature, rather close to the pylorus. In few cases has it been found in the fundus of the stomach. Many authors have emphasized the insidious onset. A large portion of the stomach may be involved without serious epigastric distress. Bleeding is a late symptom. The tumor avoids the orifices, so that obstruction is a late manifestation. Typically the tumor grows by direct extension rather than by blood-borne metastases.

D'Aunoy[2] in his review of the subject noted that the tumor may be either endogastric or exogastric and reported that the latter type is much more frequent. With respect to the histologic classifications of cases of sarcoma in the literature, he listed eighteen separate varieties

1. Lubarsch, O., and Henke, F.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1946, vol. 4; cited by Pack and McNeer.[4]

2. D'Aunoy, R., and Zoeller, A.: Am. J. Surg. 9:444, 1930.

3. Douglas, J.: Ann. Surg. 71:628, 1920.

4. Pack, G. T., and McNeer, G.: Ann. Surg. 101:1206, 1935.

of sarcoma of the stomach. The most common are: lymphosarcoma (39 per cent), round cell sarcoma (24 per cent), spindle cell sarcoma (7 per cent), fibrosarcoma (7 per cent), myosarcoma (6 per cent), round and spindle cell sarcoma (2 per cent), leiomyosarcoma (2 per cent) and fibromyosarcoma (2 per cent). He stated in a summary of the pathologic aspects of sarcoma of the stomach that despite the clinical characteristics and the bulky tumor revealed by roentgen examination, only biopsy can make the diagnosis positive. All of the authors agree that sarcoma of the stomach is frequently operable, even though the defect shown on the roentgenogram may be large.[5]

REPORT OF A CASE

M. H., a 57 year old white woman, suffered from persistent gastrointestinal discomfort for ten years before seeking medical advice in June 1945. (The clinical records made prior to her admission to the Salt Lake County General Hospital were supplied to us by Drs. R. T. Richards and V. L. Rees.) Two months earlier she had undergone thyroidectomy for a nontoxic nodose goiter. The gastrointestinal symptoms at that time consisted of epigastric pain, which was sometimes knifelike and was not relieved by food. An occasional backache was noted, and there were several bouts of emesis without blood. In October 1944 the patient noted the onset of persistent epigastric pain slightly different from the previous spasmodic episodes. This pain lasted for three weeks, and a month later there was pain in the left side of the chest. Weakness and loss of weight followed, although the diet was unchanged. In June 1945 the patient was hospitalized because of massive hematemesis. She required five transfusions. A diagnosis of carcinoma of the stomach was made, following roentgen examination. Because of the large defect and immobility of the left leaf of the diaphragm, the carcinoma was considered inoperable, and palliative treatment was instituted. The patient was ambulatory, without evidence of hemorrhage, and in fair health until May 1946, when she again became weak and bedridden. For three weeks prior to admission to the Salt Lake County General Hospital the patient had tarry stools and frequent bouts of hematemesis. One week before admission, even liquids were regurgitated, and gradual swelling of the lower extremities was noticeable. A massive loss of blood on August 11, 1946, brought the patient to the hospital a day later.

5. Zellhoefer, H. W. K.: Proc. Staff Meet., Mayo Clin. 10:625, 1935. Glenn, F., and Douglas, E.: Arch. Surg. 33: 467, 1936. Phillips, J. R., and Adam, G. F.: Am. J. Surg. 35:125, 1937. Middleton, W. S., and Paul, L. W.: Radiology 28:486, 1937. Jordan, S. M.: S. Clin. North America 18:683, 1938. Lahey, F. H.: Lahey Clin. Bull. 1:4, 1938. Leiomyosarcoma of the Stomach, Cabot Case 25082, New England J. Med. 220:351, 1939. Mass, M., and Kirshbaum, J. D.: Am. J. Roentgenol. 44:716, 1940. Horsley, G. W., and Berger, R. A.: Ann. Surg. 112:22, 1940. Ross, D. E.:Am. J. Surg. 49:503, 1940. White, R. R., and Walters, W.: Proc. Staff Meet., Mayo Clin. 16:378, 1941. Lemon, R. G., and Broders, A. C.: Surg., Gynec. & Obst. 74:671, 1942. Lyons, C. G., and Schneider, M.: Am. J. Roentgenol. 49:393, 1943. Bassler, A.: Am. J. Digest. Dis. 10:342, 1943. Schroeder, G. F., and Schattenberg, H. J.: Arch. Surg. 47:8, 1943. Chaffin, L.: West. J. Surg. 46:513, 1938. Baumgartner, C. J.: ibid. 47:27, 1939. Holta, O.: Acta radiol. 24:166, 1943.

She was extremely pale and semimoribund and was in mild shock, with a blood pressure of 85 systolic and 50 diastolic, a pulse rate of 96 and a temperature of 97.2 F. No lymphadenopathy was discovered. There was a smooth, firm, painless epigastric mass extending down to the level of the umbilicus, and on rectal examination a nodular mass was palpable in the left posterior quadrant of the abdomen. The clinical impression was that of carcinoma of the stomach, carcinomatosis and anemia. The patient rapidly became weaker, passed large amounts of tarry stools and died three days after admission despite supportive therapy.

Autopsy.—Gross Findings: At autopsy the significant features were: an elliptic healed scar over the anterior aspect of the neck, a decrease in the amount of subcutaneous fat and a palpable mass in the epigastrium. In addition there were: moderate pulmonary congestion; tumor nodules 3 to 10 mm. in diameter, in the liver; hydrops of the gallbladder; a large gastric tumor, which compressed the esophagus and was adherent to the spleen, the diaphragm and the left adrenal gland; a subserous leiomyoma of the uterus, and a serous cyst of the left ovary.

The weight of the stomach with the tumor and the distal 5 cm. of the esophagus was 1,080 Gm. The tumor measured 13 by 18 by 4 cm. and occupied the distal half of the stomach, surrounding the pylorus. It extended upward along the lesser curvature to the esophagus and appeared to infiltrate the submucosa of the esophagus, compressing the lumen. Smooth serosa-covered elevations, measuring 2.5 by 3.5 cm., were scattered over the lesser curvature and the posterior surface of the stomach. The tumor projected irregularly into the lumen, and the mucosa over the larger nodules was ulcerated. The stomach proximal to the tumor was dilated so that the fundus was 15 cm. in length and 17 cm. in circumference. Section of the tumor revealed a gray-white surface with irregular nodules of various sizes. Small soft yellow areas appeared to be necrotic, and here and there were small hemorrhages. It was assumed that the lesser curvature of the stomach was the site of origin, since it was entirely infiltrated by the tumor, while the greater curvature of the stomach was involved only at the most distal portion of the stomach. The pylorus was so narrowed that it would hardly admit a 2 mm. probe. At a point immediately proximal to the pylorus the tumor formed a huge mass that filled the lumen. No areas of hemorrhage were seen in the mucosa, and no open vessel was found which would account for the massive hemorrhage.

Microscopic Observations.—The tumor appeared to originate in the muscularis mucosae, with which it blended intimately (fig. 1). The overlying gastric mucosa was atrophic but retained its continuity. Pseudorosettes were seen, and in some places the cells were palisaded about the small blood vessels. Occasionally the nuclei were irregular and large. The characteristics of leiomyosarcoma were shown in the whorling of long, spindle-shaped nuclei (figs. 2 and 3). These cells had a moderate amount of pink-staining or red-staining cytoplasm. Some parts of the tumor showed myxomatous degeneration and large spaces filled with red blood cells.

Figure 4 shows a metastatic nodule in the liver. Here the tumor infiltrated the sinuses, causing atrophy of the liver cord cells without marked necrosis. The metastasis in this figure had the same pattern as the primary tumor.

COMMENT

Grossly, this tumor corresponds to the less frequent type of leiomyosarcoma of the stomach described by D'Aunoy as endogastric. This
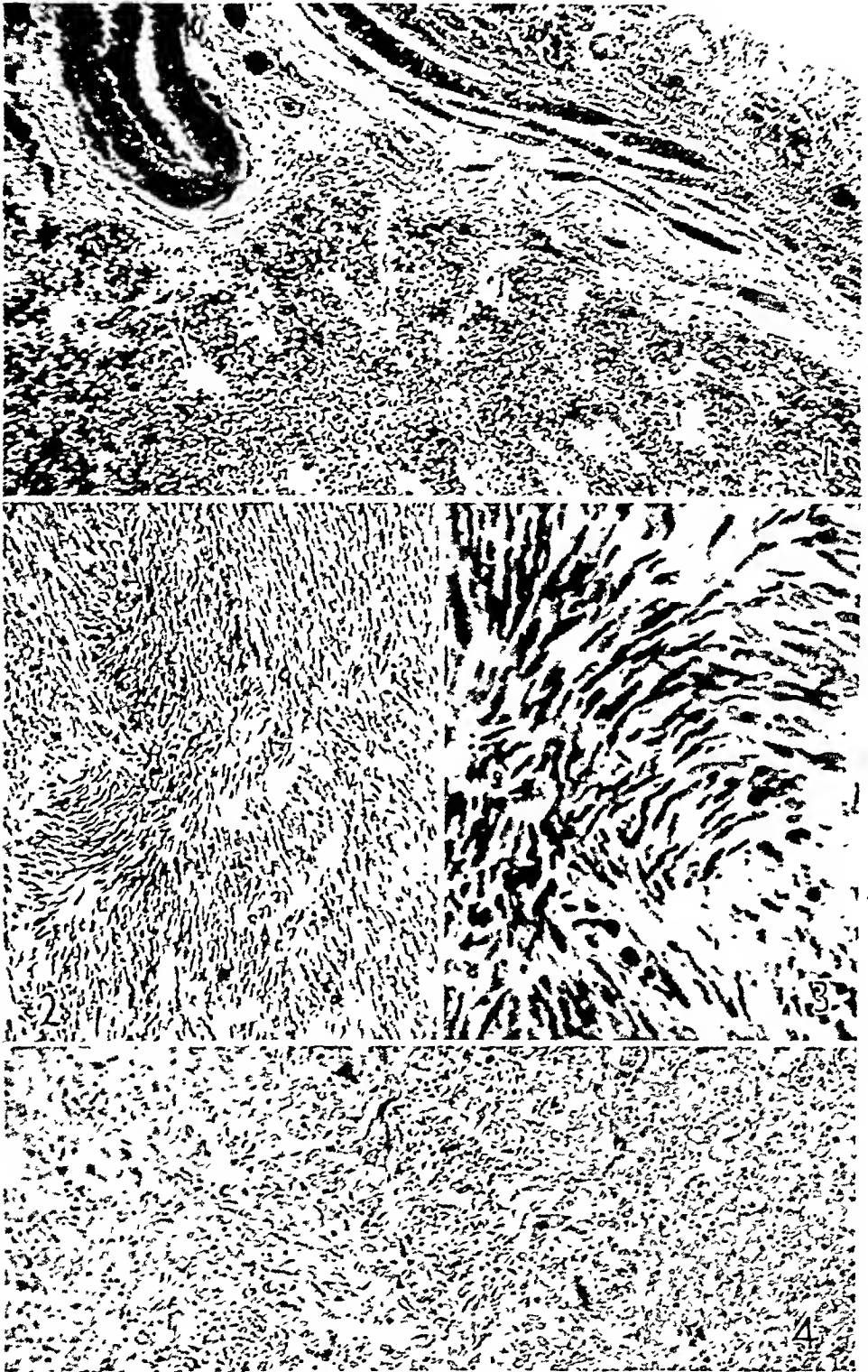
Fig. 1.—Photomicrograph showing leiomyosarcoma occupying the muscularis of the gastric wall. The mucosa is atrophic but not infiltrated by the tumor, which blends intimately with the smooth muscle (approximately × 200).

Figs. 2 and 3.—Low and high power photomicrographs of another histologic pattern of the leiomyosarcoma. The whorling effect is common in spindle cell tumors.

Fig. 4.—Hepatic metastasis. The histologic pattern is maintained (approximately × 200).

141

type has a tendency to infiltrate the wall beneath the mucosa rather than to penetrate the mucosa and produce an ulcer. After the tumor has reached a certain bulk it projects into the lumen. This particular tumor caused partial pyloric obstruction, although this is unusual for sarcoma of the stomach. The leiomyosarcoma is characteristically formed on the lesser or the greater curvature of the stomach and rarely involves the cardia. This tumor produced the typical clinical syndrome of hematemesis and shock. Here again the hematemesis is seen to be undoubtedly a late clinical manifestation. It is presumed that this tumor had been growing for a long time. Symptoms of the disease appeared two years before death, and it is assumed that the tumor grew for some time before producing any symptoms.

As to the histologic structure of the tumor, we wish to emphasize that several cytostructural variations may be seen in leiomyosarcoma. In one area whorling of the nuclei may be prominent; in another degenerative changes may predominate, with necrosis, hemorrhage and myxomatous changes; in other regions pseudorosette formation may be accentuated, or there may be marked variations in the size and shape of nuclei with giant cell formation and many mitotic figures. Therefore, we feel that the bizarre sarcomatous appearances in the several sections from this tumor represent only variations of one type of tumor, that is, leiomyosarcoma. The various histologic descriptions given in reports of cases in the literature add to the confusion of the microscopic picture, and it might be well to call all of these cases instances of leiomyosarcoma from the demonstrated origin of the tumor in smooth muscle and to speak of other variants as degeneration or predominant cell types. Neurosarcoma and lymphosarcoma have not been mentioned. It is believed that each of these tumors is of a separate origin, with a distinct pathologic pattern of its own.

SUMMARY

A case of leiomyosarcoma of the stomach with hepatic metastases is reported. The chief gross characteristic of the tumor was the infiltration of the muscularis mucosae with minimum ulceration of the mucosa. The histologic structure included spindle-shaped nuclei, whorling of cells, pseudorosette formation, irregularity of the size of the nuclei and degenerative changes. The confusion of the histologic nomenclature is discouraged, and the term "leiomyosarcoma of the stomach" is preferred for tumors of this type.

RELATIONSHIP OF GROWTH AND NUTRITION TO CARDIORENAL CHANGES INDUCED IN BIRDS BY A HIGH SALT INTAKE

CECIL A. KRAKOWER, M.D.

AND

HELEN E. HEINO, M.S.

CHICAGO

SODIUM CHLORIDE administered to young, rapidly growing chicks in excess of the permissible 2 per cent of the food intake produces hydrops and cardiac and renal hypertrophy.[1] There is marked enlargement of the renal glomeruli, which is due not only to symmetric growth of the core and the capillaries but to new formation of tufts or papillae.[1c] These arise as papillary outgrowths from the nucleated core, carrying the overlying capillaries with them. Abundant reticulum is deposited within the periphery of such enlarged glomeruli and particularly within the newly formed papillae. The visceral epithelium over the glomerulus proliferates and hypertrophies, with accompanying hyaline and fatty degenerative changes. Renal interlobular arteries and arterioles become tortuous and thick walled but are free from any form of sclerosis.

It was noted in earlier experiments that these morphologic changes appeared to vary with the nutritional status of the chick and with its age at the time when it was first treated with salt. There were two important factors which had to be considered with regard to these variations: (1) the rate of growth of the animal; (2) the effect of an increasing load of salt and water such as is imposed on the rapidly growing bird as a result of the progressive increase of the daily food intake with a fixed concentration of sodium chloride. The following experiments were therefore designed to correlate growth and nutrition with salt and water load in an attempt to determine to what extent each of these factors, or all combined, influence the described cardiorenal changes.

PROCEDURE

One day old New Hampshire chicks were purchased and maintained on a chick starting mash in electrically heated brooders for the first three weeks.

Thereafter they were placed in individual screened cages and fed a chick growing mash.[2]

In the first five groups of table 1 female chicks were used exclusively. Those in the first four groups were restricted to the experimental diets when they were 28 to 32 days of age. In group 5 the experimental birds had been primed on 3 per cent salt starting mash for the last two weeks in the brooders, before they were given 5 per cent salt growing mash when they were 3 weeks old. A small number of 29 day old cockerels were employed in group 6 and adult cocks 6 months old in group 7.

The composition of the diets fed daily to the different groups of birds is summarized in table 1. Each diet was so constructed as to allow us:

(1) To distribute the salt and water load equally during the day by adding requisite amounts of Cellu Flour.[3] This tended to simulate the eating habits of birds fed ad libitum while permitting us at the same time to maintain a constant concentration of sodium chloride despite the reduced intake of food.

(2) To determine the effect of the daily ingestion of a fixed amount of salt at different caloric levels.

(3) To determine the effect of the daily ingestion of an increasing amount of salt at different caloric levels.

(4) To determine the effect of the concentration of salt in the diet. The concentration of sodium chloride had perforce to be increased in group 2C in which the birds could eat little more than 25 Gm. of the mixture daily and in group 7B, in which adult birds ate a fairly constant amount of food daily. On the other hand, in groups 3B and 6B the concentration of salt was allowed to decrease, although the amount ingested daily was kept constant.

The consumption of food and water was recorded daily. In the determinations of consumed water the amounts lost by evaporation were corrected for. The birds were weighed twice weekly. Food and water were withdrawn from the birds fifteen to eighteen hours prior to the termination of the experiment. The animals were weighed, then anesthetized with chloroform, and blood was withdrawn from the heart. With the blood so obtained, hemoglobin determinations ("Cenco-Sheard-Sanford photelometer"), hematocrit readings (Wintrobe tubes) and chemical analyses were made. The birds were killed immediately after the withdrawal of blood and dissected. In removing the heart, the aorta was regularly sectioned at the level of the junction of the ascending and the transverse portions of the arch. If the epicardium was edematous, multiple incisions were made through it to allow the fluid to escape. The chambers of the heart were opened and emptied of blood. Before the organ was weighed, the epicardial and endocardial surfaces were carefully blotted with filter paper. Before the kidneys were weighed, their external surfaces were likewise blotted dry. To obtain dry weights, the heart

---

2. These mashes were obtained from the Ralston Purina Mills, St. Louis. The composition of the growing mash ("purina chick growena") is given as protein 17 per cent, fat 3 per cent, nitrogen-free extract 48 per cent and crude fiber 7 per cent, yielding an estimated 388 calories per hundred grams of food. It contains 0.5 per cent iodized salt.

3. Cellu Flour is basically cellulose and has no caloric value. It is supplied by the Chicago Dietetic Supply House, Inc., Chicago.

TABLE 1.—*The Composition of the Daily Diet*

Group	Growing Mash, Gm.	"Cellu Flour," Gm.*	NaCl, Gm.†	Duration Prior to Change, Days	Caloric Value
1 A	6.0	18.0	...	6	23.2
	5.9	17.6	...	..	22.8
B	6.0	18.0	1.0 (4%)	6	23.2
	5.9	17.6	1.5 (6%)	..	22.8
2 A	12.0	11.5	...	..	46.5
B	12.0	11.5	1.5 (6%)	..	46.5
C	12.0	11.5	1.5 (6%)	6	46.5
		| decrements ↓ of 0.3	| increments ↓ of 0.3	| consecutive ↓ 4 day periods	
	12.0	10.0	3.0 (12%)	..	46.5
3 A	18.0	5.5	...	6	69.8
	18.0	12.0	...	4	69.8
		| increments ↓ of 5.0		| consecutive ↓ 4 day periods	
	18.0	32.0	...	..	69.8
B	18.0	5.5	1.5 (6%)	6	69.8
	18.0	10.5	1.5 (5%)	4	69.8
		| increments ↓ of 5.0	| ↓	| consecutive ↓ 4 day periods	
	18.0	30.5	1.5 (3%)	..	69.8
C	18.0	5.5	1.5 (6%)	6	69.8
	18.0	10.2	1.8 (6%)	4	69.8
		| increments ↓ of 4.7	| increments ↓ of 0.3	| consecutive ↓ 4 day periods	
	18.0	29.0	3.0 (6%)	..	69.8
4 A	23.5	....	...	..	91.1
B	23.5	....	1.5 (6%)	..	91.1
C	23.5	....	1.5 (6%)	6	91.1
	23.5	4.7	1.8 (6%)	4	91.1
		| increments ↓ of 4.7	| increments ↓ of 0.3	| consecutive ↓ 4 day periods	
	23.5	23.5	3.0 (6%)	..	91.1
5 A	Ad lib. av. 34.1	......	....	....	Av. 132.3
B	Ad lib. av. 35.8	......	Av. 1.8 (5%)	....	Av. 138.9
6 A	Progressive increase from 30 to 95	......	....	Over total period of 35 days	116.4-368.6
B	Progressive increase from 28.5 to 93.5	......	1.5 (5% decreasing to 1.5%)	Over total period of 35 days	110.5-362.7
7 A	Ad lib. av. 145	......	....	Over total period of 38 days	562.6
B	Ad lib. av. 137.2-98.2	......	Av. 2.8 (2%) 13.4 (12%)	Over total period of 38 days	Av. 532.3-381.0

* See footnote 3, page 2.
† Figures in parentheses represent the concentration of salt.

and the kidneys were kept in an oven at 100 C. until their weights were constant. Representative sections of all organs were fixed both in Zenker's fluid and in 10 per cent formaldehyde solution.

<center>RESULTS</center>

Percentage Loss or Gain in Weight: Daily Salt and Water Intake.— The pertinent data on the first five groups of animals are given in table 2. The percentage loss of weight in group 2 approached that of group 1 despite the fact that the caloric intake in the former was double that of the latter, but this is readily explained by the longer survival period of the birds on the higher "caloric diet." In the same way, the weight gain of the birds given food ad libitum (group 5) would have been appreciably higher if the experiment had not been terminated a week or two earlier than in groups 3 and 4. The loss of body weight was on the whole greater and the gain of weight less in the experimental animals as compared with their respective controls. This was particularly true of those animals on an ascending scale of salt intake. The fact that these differences are not too apparent in the first two groups can best be accounted for by the presence of edema fluid in the experimental animals which could not be corrected for by employing the lowest weight recorded prior to demonstrable ascites or anasarca.

As a basis for comparison of the effects of high salt intake at different nutritional levels, it will be noted that there was more than a 30 per cent loss in body weight over a two to three week period in the first group of birds, a loss of about 25 per cent over a four week period in the second group, maintenance of the original body weight or stability of weight in the third group, a slow increase in weight over a five to six week period in the fourth group and a normal rapid rise in the fifth group.

A uniform salt load was obtained in all five groups when it was expressed as an average of the daily salt intake in grams per hundred grams of body weight. In those on a fixed daily intake of salt it varied within the narrow range of 0.46 to 0.54 Gm., whereas in those on an ascending scale of salt it varied from 0.68 to 0.74 Gm., which only slightly exceeded the value of 0.65 Gm. obtained in birds on 5 per cent sodium chloride fed ad libitum. It is of interest that the birds in group 6 (table 1) that were allowed to grow freely and were given 1.5 Gm. of sodium chloride daily reduced this daily load from 0.42 Gm. per hundred grams of body weight at the beginning of the experiment to 0.17 at its end. Such a low level, as will be indicated in the section on histopathology, is ineffective in either producing or maintaining the characteristic changes observed in rapidly growing chicks and was in fact associated with regression of the lesions produced earlier by the higher percentage level. In the case of the fully grown cocks in group 7 B (table 1), on the other hand, the value for the average daily salt intake per hundred grams of body weight rose from 0.21 Gm. when

Table 2.—*Loss or Gain in Body Weight and Daily Salt and Water Intake*

Group	Diet	Birds	Age at Onset of Experiment, Days	Duration of Experiment, Days	Initial Weight, Gm.*	Final Weight, Gm.*	Loss or Gain in Weight, %	Av. Daily Salt Intake, Gm.† / Body Weight, Gm.	Av. Daily Water Intake, Cc.‡ / Body Weight, Gm.
1A	6.0 Gm. (control)	8	28	16-20	238.5±22.8	160.1±7.9	−30.7±3.6	....	42.3
1B	6.0 Gm., fixed salt intake	9	28	11-17	250.2±27.5	180.0±21.2	−30.5±4.6	0.49	61.7
2A	12.0 Gm. (control)	6	32	21-32	288.7±31.9	207.7±21.0	−27.6±6.3	....	37.9
2B	12.0 Gm., fixed salt intake	6	32	18-23	292.8±33.2	223.6±30.4	−23.8±1.9	0.51	69.0
2C	12.0 Gm., ascending salt intake	6	32	15-27	314.6±13.5	227.6±11.1	−27.9±3.6	0.74	84.0
3A	18.0 Gm. (control)	6	33	26-32	298.5±31.1	282.7±35.8	− 4.2±11.0	....	31.1
3B	18.0 Gm., fixed salt intake	6	33	23-32	311.3±23.0	302.6±11.5	− 1.1±8.1	0.16	60.0
3C	18.0 Gm., ascending salt intake	6	33	20-30	311.1±24.1	283.1±38.0	− 7.5±19.5	0.03	83.8
4A	23.5 Gm. (control)	6	30	31-40	251.8±27.1	375.0±31.1	+ 49.7±13.9	....	30.2
4B	23.5 Gm., fixed salt intake	7	30	31-10	271.1±43.0	391.7±52.4	+ 43.5±8.3	0.18	67.1
4C	23.5 Gm., ascending salt intake	7	30	31-10	273.6±21.1	343.3±30.1	+ 25.1±5.0	0.72	59.8
5A	Ad lib. (control)	6	21	21	180.3±16.9	391.8±63.1	+117.8±21.1	....	26.2
5B	Ad lib., 5% salt	6	21	21	207.3±20.6	381.0±65.3	+ 85.2±28.5	0.05	63.7

* When possible and necessary, the weights were corrected for edema by employment of the lowest weight prior to demonstrable ascites or anasarca.
† The salt referred to is that daily added to the food over and above the 0.5 per cent already present.
‡ The ratio is expressed in percentage.

147

they were on 4 per cent salt to 0.42 Gm. on 12 per cent salt. The latter is more or less equivalent to that ingested by birds with a fixed daily salt intake but restricted in growth. No attempt was made to increase the concentration of sodium chloride in the food beyond 12 per cent in order to match that of the groups on an ascending scale of salt ingestion, inasmuch as food consumption diminished pari passu with increased concentration. This is apparent from table 1 where the food consumption dropped from 137.2 Gm. daily in the early experimental periods to 98.2 Gm. toward the end. It was our intention to maintain these animals at a stable weight and on an adequate intake of food for comparison with group 3 particularly.

Water consumption closely paralleled salt intake. In day by day values and particularly in certain birds in the subgroups on an ascending scale of sodium chloride, the amount of water ingested daily often greatly exceeded the animal's body weight. Although the birds in group 5B, fed an ad libitum diet, consumed as much salt per day as the birds on a reduced caloric intake but with increasing levels of salt, their consumption of water paralleled that of the birds on a fixed daily intake of salt. The reason for this is not clear.

Hemoglobin Values, Hematocrit Readings and Results of Chemical Analysis of Blood.—These values for groups 2 to 5 inclusive are given in table 3. It is of interest that with regard to both range and average the concentration of hemoglobin was essentially the same in all control groups despite starvation. In those given sodium chloride, however, there was a wide range in groups 2 to 4. The very low values for hemoglobin in groups 2 B, 2 C and 3 C are in great part ascribed to profuse intestinal hemorrhage, while the very high values are probably due to dehydration and in some instances to shock. The latter was seen either in association with massive hemorrhage of the intestinal tract or in birds with marked anasarca. Aside from these factors there is little to indicate that high intake of salt per se was instrumental in producing anemia. In fact, despite the marked gelatinous and edematous character of the bone marrow of birds that were rapidly declining in weight, there always appeared to be a little more hemopoietic tissue microscopically in the treated animals than in the controls. It was markedly reduced in both, however. The bone marrow in birds that were maintained at a stable weight in group 3 was still edematous and hypoplastic. In all others it was essentially of normal fatty character, with greater cellularity and activity in treated animals.

The values for nonprotein nitrogen of the blood were practically all within the normal range. The few exceptions were found in the groups whose intake of food was restricted, with the two highest values in those given 12 Gm. of food daily, viz., 60 mg. per hundred cubic centimeters

for a control and 94 mg. for one on an ascending intake of salt. The latter was moribund when it was killed, and there was considerable blood in its intestinal tract. Probably in these later stages of starvation cardiovascular failure, with or without ascites, tended to reduce renal filtration, accounting for the elevated level of nitrogenous products in the blood of some of the birds. It is doubtful whether blood absorbed from the intestinal tract could account for it in most of these cases since, in their debilitated state, the birds died soon after such profuse hemor-

TABLE 3.—*Hemoglobin Values, Hematocrit Readings and Results of Chemical Analyses of Blood*

Group	Diet	Birds	Hemoglobin, Gm. per 100 Cc.	Hematocrit Reading, Vols. %	Blood Nonprotein Nitrogen, Mg. per 100 Cc.	Plasma Protein,[†] Gm. per 100 Cc.	Plasma Chlorides, Mg. of NaCl per 100 Cc.
2A	12.0 Gm. (control)...............	6	8.3-10.8* / 9.4	29-36 / 32.5	12.5-60.0 / 31.3	0.04-1.7 / 0.83	635-725 / 685.8
2B	12.0 Gm., fixed salt intake.......	4	4.5-10.8 / 7.7	15-34 / 25	16.5-40.0 / 28.3	0.17-0.99 / 0.64	695-840 / 746
2C	12.0 Gm., ascending salt intake..	3	5.1-8.8 / 7.1	16-30 / 23.6	9.3-94.0 / 39.7	0.50-1.38 / 0.84	760-900 / 850
3A	18.0 Gm. (control)...............	6	9.1-9.6 / 9.3	29-34 / 32	26.4-49.5 / 32.3	0.82-2.02 / 1.34	710-750 / 732
3B	18.0 Gm., fixed salt intake.......	5	6.8-12.7 / 10.3	22-40 / 30.6	12.5-26.4 / 19.9	1.39-2.87 / 2.00	680-750 / 706
3C	18.0 Gm., ascending salt intake..	4	2.0-12.3 / 8.9	8.0-42.5 / 29.8	9.9-50.0 / 33.5	0.76-2.72 / 1.95	600-880 / 742.5
4A	23.5 Gm. (control)...............	5	9.1-10.5 / 9.9	30-37 / 32	24.7-32.5 / 28.1	1.80-3.54 / 2.56	
4B	23.5 Gm., fixed salt intake.......	5	6.4-9.9 / 8.6	.....	13.9-30.4 / 22.0	1.31-2.76 / 2.23	
4C	23.5 Gm., ascending salt intake..	6	7.6-11.2 / 9.6	.....	24.7-46.0 / 31.9	2.50-4.86 / 3.36	
5A	Ad lib. (control)................	6	7.6-10.8 / 9.3	30-36 / 32.8	23.1-39.3 / 27.4	2.20-3.88 / 2.88	
5B	Ad lib., 5% salt................	10	8.3-10.5 / 9.6	30-37.5 / 33.8	18.5-33.3 / 25.3	2.05-4.27 / 2.96	

* In this and the following columns the top figure represents the range; the lower single figure, the average.
† Method of Campbell and Hanna, modified (J. Biol. Chem. **119**: 15, 1937).

rhage. It is of interest, however, that despite the marked glomerular changes in the treated animals fed ad libitum, there was no evidence of nitrogenous retention.

The plasma proteins graded themselves in accordance with the daily caloric intake. The lowest values in the four groups in which they were determined were obtained in birds given 12 Gm. of food daily. These low values were, however, unassociated with ascites in the controls (table 6), though frequently accompanied by accumulations of such fluid in the treated animals. The same was true to a certain extent for the group fed 18 Gm. of food daily. It is a striking fact that values as low as 0.04 Gm. and 0.27 Gm. per hundred cubic centimeters were unassoci-

·ated with edema in the controls, while appreciably higher values were associated with edema in salt-treated animals. It ˉseems also true that in general the plasma proteins were higher in salt-treated than in control animals of all groups, as though a compensatory mechanism had been brought into play whereby plasma proteins were produced to a greater degree or mobilized more freely.

Hyperchloremia occurred chiefly in the subgroups on ascending intake of salt and was more pronounced in the subgroup given 12 Gm. of food daily than in the one given 18 Gm. In some but not all of these instances there was nonprotein nitrogen retention as well as hyperchloremia, strengthening the view already expressed regarding cardiovascular failure and reduced renal filtration.

Blood Pressure and Heart Rates.—The determinations of arterial blood pressure were performed by Mr. W. Glen Moss, of the department of physiology of the University of Illinois. A modified Hamilton manometer was employed. Through a small incision in the unanesthetized bird, the femoral artery was exposed, and direct intra-arterial blood pressure readings were obtained. In view of the relatively small size of the artery, narrow-gaged needles had to be used, which yielded records from which mean pressures could be determined but not systolic or diastolic readings.

It will be seen from table 4 that both young and older birds were employed under a variety of conditions. Young chicks 24 days of age were fed 12, 18 or 23.5 Gm. of food daily, which resulted in loss of body weight, maintenance of body weight or slow gain in weight respectively. Their experimental counterparts were fed sodium chloride on an ascending scale in a manner similar to that outlined in table 1. As far as the young adult birds were concerned, some were fed 20 or 35 Gm. of food daily, with marked loss of body weight at the former level and approximate maintenance of body weight on 35 Gm. The treated animals were retained on 5 per cent sodium chloride.

The findings in 51 birds, comprising a total of 87 observations, are recorded. The average mean pressures were consistently higher in almost all groups of birds on salt than in their respective controls. The greatest differences or highest pressures, however, were obtained in treated young chicks which were allowed to grow slowly, in young adult birds given food ad libitum with 5 and 8 per cent salt and in treated adults on a maintenance diet. It will be noted that there was a lesser decline of blood pressure in treated birds that· were sharply restricted in their caloric intake as compared with their controls. Excessive concentration of salt, as in the case of adults on 15 per cent sodium chloride, tended to depress the blood pressure in the same manner as undernutrition.

It is of interest that with regard to heart rates the situation, at least in young birds, was reversed. The rate tended to be slower in treated than in control animals. The differences were, however, slight in adults inasmuch as the heart rate is normally slower in older birds.

TABLE 4.—*Blood Pressure Determinations and Heart Rates*

Diet	Age of Birds, Days	Birds	Observations	Days on Diet	Mean Blood Pressure, Mm. Hg *	Heart Rate per Minute *
12 Gm. food + 11.5 Gm. "cellu flour"	24	4	6	5-14	82-128 112.1	314-564 458
12 Gm. food + ascending 6.0-8.4% NaCl + "cellu flour"	24	3	5	5-12	95-135 114.2	175-435 332
18 Gm. food + ascending "cellu flour"	24	2	4	5-12	95-124 114.2	348-460 352
18 Gm. food + 6% ascending NaCl + ascending "cellu flour"...	24	3	5	5-12	108-140 121.8	254-456 367
23.5 Gm. food + ascending "cellu flour"	24	2	4	5-12	100-138 114.0	438-464 456
23.5 Gm. food + 6% ascending NaCl + ascending "cellu flour"..	24	2	4	5-12	108-145 132.5	344-440 393
Ad lib. (control)	32-38	2	4	....	106-122 113.2	444-486 471
Ad lib. + 3% NaCl	32-38	4	6	On NaCl from 1 week of age	95-130 113.0	376-486 440
Ad lib. + 5% NaCl	32-38	4	5	On NaCl from 1 week of age	100-135 120.0	346-414 378
Ad lib. (control)	90 and up	7	13	....	112-132 126.6	300-440 363
20 Gm. food + 20 Gm. "cellu flour"	90 and up	2	4	34-48	96-105 99.7	300-360 332
35 Gm. food + 25 Gm. "cellu flour"	90 and up	3	5	30-48	75-122 99.0	330-360 312
Ad lib. + 5% NaCl	90 and up	5	9	On NaCl from 1 week of age	116-168 143.1	270-435 367
Ad lib. + 8% NaCl	90 and up	2	2	On NaCl from 1 week of age	150, 150 150	348, 396 372
Ad lib. + 15% NaCl	90 and up	2	4	On NaCl from 1 week of age	105-142 123.7	240-390 315
20 Gm. food + 5% NaCl + 18 Gm. "cellu flour"	90 and up	1	1	On NaCl from 1 week of age; on restricted diet for 34 days	 115	 95
35 Gm. food + 5% NaCl + 22 Gm. "cellu flour"	90 and up	3	6	On NaCl from 1 week of age; on restricted diet for 30-48 days	125-162 139.1	240-330 295

* The top figure represents the range; the single lower figure, the average.

Gross Anatomic Findings.—Despite the profound emaciation of the birds on 6 or 12 Gm. of food daily, there was little anatomic evidence of any specific vitamin deficiency. In some birds fed these restricted diets,

however, plantar hyperkeratoses were present, which ulcerated and bled. This may have indicated a deficiency of biotin or of pantothenic acid. It is also possible that the intestinal hemorrhages so often found in these starved birds might have been due in part to vitamin K deficiency, aside from starvation. Hemorrhages elsewhere, however, were practically never observed. Erosions of the gizzard were rarely seen. Osteoporosis was an almost constant finding in these two groups of birds and occurred occasionally in birds fed 18 Gm. of food daily. Edema of the epicardial tissues with absence of fat was constantly observed in birds fed 6, 12 and 18 Gm. of food, respectively, and not infrequently in those fed 23.5 Gm. of food daily. The degree of epicardial edema was more marked in treated than in control animals.

TABLE 5.—*Gross Autopsy Findings*

Group	Diet	Birds	No. that Died	No. with Intestinal Hemorrhage	No. with Ascites	No. with Hydropericardium
1A	6.0 Gm. (control)...............	8	3	2	1	2
1B	6.0 Gm., fixed salt intake.......	9	5	2	4	5
2A	12.0 Gm. (control).............	6	..	3	..	1
2B	12.0 Gm., fixed salt intake......	6	1	1	4	6
2C	12.0 Gm., ascending salt intake	6	2	3	4	4
3A	18.0 Gm. (control).............	6	..	2	..	..
3B	18.0 Gm., fixed salt intake......	6	1	1	2	4
3C	18.0 Gm., ascending salt intake	6	1	2	2	4
4A	23.5 Gm. (control).............	6	..	..	..	..
4B	23.5 Gm., fixed salt intake......	7	..	..	..	3
4C	23.5 Gm., ascending salt intake	7	..	..	1	3
5A	Ad lib. (control)...............	6	..	..	..	..
5B	Ad lib., 5% salt................	6	..	..	..	3
7A	Adult cocks (controls).........	3	..	..	..	..
7B	Adult cocks on ascending salt intake......................	3	..	..	..	1

It is apparent from table 5 that the mortality was particularly high in group 1, diminishing in groups 2 and 3. There was none in the remaining groups. The mortality was greater in treated than in control animals of the first three groups.

Intestinal hemorrhage was observed in the first three groups on 6, 12 and 18 Gm. of food, respectively, and with equal frequency in control and experimental birds. It was either slight or, more often, profuse. The bleeding occurred from multiple punctate areas in the duodenum and upper portions of the small intestine. This seemed to be preceded by erythema of these areas of mucosa as contrasted with the pallor of the mucous membrane elsewhere.

The incidence of ascites was particularly high (66 per cent) in the salt-treated animals given 12 Gm. of food, but occurred in 44 per cent of the birds in group 1B and in 33 per cent of those on either fixed or

ascending levels of salt with an intake of 18 Gm. of food daily. By contrast with experiences in earlier experiments in which hydrops occurred fairly frequently in birds given food ad libitum with 3 per cent sodium chloride, no edema developed in group 5B given a 5 per cent salt diet ad libitum. The better quality of the commercial feed used in these experiments and the season of the year when the experiment was performed have probably had a good deal to do with this. With shorter hours of daylight there appears to be less tendency for anasarca to develop in birds with a high salt intake fed ad libitum than with longer hours of daylight in summer. This may be due to overloading as a result of longer feeding time.

It will be noted, however, that hydropericardium was the commonest gross anatomic finding in all groups of birds on salt.

Heart and Kidney Weights.—The heart weight per unit of body weight was appreciably higher in treated birds as compared with their respective controls in all experiments (table 6). This enlargement was due mainly to increased ventricular capacity with maintenance of, or a variable degree of increase in, myocardial thickness of both right and left ventricles. Statistical analysis reveals that the cardiac hypertrophy was highly significant in all groups except those fed 6 and 12 Gm. of food, respectively. The degree of hypertrophy varied from 31.0 to 62.2 per cent but within these four groups (3, 4, 5 and 7, table 6) was independent of the caloric intake, the salt level or the age of the birds. In the case of the birds given 6 and 12 Gm. of food daily, the heart weights of the treated animals exceeded those of the controls by 16.1 and 18.6 per cent, respectively, as computed on the basis of heart weight–body weight ratios. Nevertheless, from a statistical standpoint the differences in these two groups were not too significant. They do indicate, however, that despite profound loss in body weight, cardiac weight tends to be retained in the animals to which salt has been administered, while it decreases appreciably in the controls. There is no hypertrophy in a strict sense since the average cardiac weights of these experimental animals approximated the weights estimated at the onset of the experiment prior to a loss of 25 per cent or more of body weight.

It was quite different when kidney weights were analyzed statistically. Highly significant differences between experimental and control values were obtained only in birds having 18 Gm. of food and a fixed salt intake and in young pullets and adult cocks fed ad libitum. It is of interest, however, that there was a definite trend in any single group whereby the degree of renal enlargement varied directly with the daily salt intake; i. e., there was a greater degree of renal enlargement in birds on an ascending scale of salt than in those on a fixed daily intake

of salt. In terms of percentage increase in renal weight above their respective controls, those on a fixed salt intake ranged from 11.8 to 34.3, and those on an ascending salt intake, from 27.6 to 55.2. Here, too, the renal enlargement was relative in birds on 6 and 12 Gm. of food daily and on a fixed intake, the weights being approximately equal

TABLE 6.—*Kidney and Heart Weights*

Group	Diet	Birds	Heart Weight * / Body Weight	Dry Heart Wt. * / Wet Heart Wt.	Kidney Weight * / Body Weight	Dry Kidney Wt. * / Wet Kidney Wt.
1A	6.0 Gm. (control)	7	0.62±0.07 0.028	....	0.92±0.14 0.055	
1B	6.0 Gm., fixed salt intake	4	0.72±0.07 0.035 0.1>P>0.05	....	1.12±0.14 0.071 0.1>P>0.05	
2A	12.0 Gm. (control)	6	0.59±0.03 0.014	17.6±0.6	0.76±0.07 0.031	21.5±0.7
2B	12.0 Gm., fixed salt intake	5	0.70±0.12 0.054 0.1>P>0.05	16.2±0.2 (3 birds)	0.85±0.16 0.074 0.4>P>0.3	19.6±0.9 (3 birds)
2C	12.0 Gm., ascending salt intake.	5	0.70±0.08 0.038 0.05>P>0.02	16.6±1.6 (3 birds)	0.98±0.22 0.100 0.1>P>0.05	19.8±1.4 (3 birds)
3A	18.0 Gm. (control)	6	0.53±0.08 0.034	17.4±0.7	0.68±0.11 0.046	21.7±0.6
3B	18.0 Gm., fixed salt intake	6	0.86±0.15 0.063 P<0.01	17.4±1.3 (5 birds)	0.91±0.10 0.041 P<0.01	21.0±1.2 (5 birds)
3C	18.0 Gm., ascending salt intake.	6	0.85±0.15 0.063 P<0.01	16.9±1.4 (4 birds)	1.05±0.37 0.154 0.1>P>0.05	19.4±2.6 (4 birds)
4A	23.5 Gm. (control)	6	0.58±0.05 0.020	21.6±1.9	0.70±0.22 0.091	
4B	2.35 Gm., fixed salt intake	7	0.76±0.11 0.043 P<0.01	21.6±2.9	0.88±0.25 0.095 0.3>P>0.2	
4C	23.5 Gm., ascending salt intake.	7	0.85±0.15 0.057 P<0.01	19.0±1.0	1.07±0.22 0.085 0.02>P>0.01	
5A	Ad lib. (control)	6	0.59±0.05 0.022	....	0.86±0.06 0.027	
5B	Ad lib., 5% salt	6	0.89±0.17 0.070 P<0.01	....	1.08±0.12 0.047 P<0.01	
7A	Adult cocks (controls)	3	0.45±0.02 0.012	17.7±1.0†	0.41±0.02 0.014	22.9±1.1
7B	Adult cocks on ascending salt intake	3	0.62±0.11 0.064 P<0.01	20.3±0.5†	0.52±0.10 0.060 P<0.01	21.8±0.4

* The ratio is expressed as a percentage. The top figure represents the mean ± the standard deviation of the mean $\left[\sigma = \sqrt{\dfrac{\Sigma d^2}{n\text{-}1}} \right]$.

The single lower figure represents the standard error of the mean $\left[S\hat{E}m = \dfrac{\sigma}{\sqrt{n}} \right]$.

The P values were obtained from Fisher's tables.
 † These determinations are on a fat-free basis.

to those estimated for their given body weights at the onset of the experiment, whereas there appeared to be actual hypertrophy in those on an ascending salt intake and 12 Gm. of food.

The differences in dry weights between control and experimental hearts and kidneys were sufficiently insignificant to alter but little the differences in the wet weight of the organs as described.

There were only 4 animals in group 6 (table 1). The average heart weight–body weight percentage of the two controls was 0.46 and that of the two experimental birds 0.60. There was no difference in the kidney weight–body weight percentage of the two groups, which averaged 0.70. Cardiac hypertrophy seemed to be retained in the experi-

TABLE 7.—*Comparison of Renal Microscopic Changes in Experimental Birds and Appropriate Controls**

Group	Diet	Glomerular Size	Glomerular Tufting †	Glomerular Reticulosis ‡	Hypertrophy and Hyperplasia of Visceral Epithelium of Glomeruli	Arterial and Arteriolar Hypertrophy
1	6.0 Gm. food...................	0	0	0	0	1
2	12.0 Gm. food daily..........	0 (1)	0 (1)	1-2	1 (3)	1 (4)
3	18.0 Gm. food daily..........	0-2	0 (1)	1-2	1	2-3
4	23.5 Gm. food daily..........	0-1	0 (1)	1-2	1	1-3
5	Ad lib.......................	4	4	2 4	4	4
6	Progressive increase in food intake, with fixed daily salt intake in experimental animals......................	1	1	1	1	1
7	Adult cocks, fed ad lib., with ascending salt intake in experimental animals.....	0-3	0	1-3	4	2-3

* The microscopic changes are graded as follows: 0 signifies no change; 1, slight increase; 2, moderate increase; 3, marked increase; 4, very marked increase. The figures in parentheses represent changes found in an occasional bird but not characteristic of the group.

† Glomerular tufting refers to the new tufts or papillae formed as outgrowths from the core.

‡ Glomerular reticulosis refers to the increased deposition of reticulum noted beneath and between the glomerular capillaries as well as to some extent within the glomerular core itself.

mental animals with little evidence of regression despite the fact that the salt intake per unit of body weight was reduced below 0.2 per cent.

Microscopic Observations.—There was profound visceral and skeletal muscular atrophy in the first two groups of birds on 6 and 12 Gm. of food daily. Muscular dystrophy was encountered in 2 birds, a control on 12 Gm. and a treated animal on 18 Gm. of food and a fixed salt intake. Aside from these there were few findings of note other than those in the kidneys. The changes in arterial vessels detailed in following paragraphs were much less apparent in other organs or tissues of experimental animals. It may be added, however, that in exposing the femoral arteries for determinations of blood pressure we observed that these vessels were wider and fuller in the experimental than in the control animals.

Table 7 presents the changes in glomeruli and renal blood vessels of treated animals, whether these were on fixed or on ascending ·salt levels, as compared with their respective controls in each of the seven groups outlined in table 1. These changes in the structures referred to pertain to the inner third of the renal cortex and were evaluated qualitatively. It was only in adult birds that the glomeruli of the outer two thirds of the cortex participated in any way in these changes.

Increase in glomerular size was most pronounced in actively growing birds of group 5 (fig. 6). It was slight in those of group 6 which were allowed to grow freely but in which the salt intake per unit of body weight was allowed to decline below 0.2 per cent. In all other groups there was either no change in size, as in those on sharply restricted caloric intakes,. or slight to moderate change in the others, with the exception of more marked hypertrophy in one of the adult cocks on an ascending scale of salt (fig. 8).

Glomerular tufting, or papillary outgrowth from the glomerular core, was confined almost entirely to group 5 (fig. 6). There was some persistent tufting of the glomeruli in group 6 in association with the ·remolding of the glomerulus by growth alone, inasmuch as the stimulus induced by salt was largely ineffective in maintaining or augmenting the hypertrophied state produced in the earlier stages of the experiment when the salt intake per unit of body weight was greater than 0.2 per cent. In all other groups, whatever glomerular enlargement occurred was symmetric in character (figs. 2 and 8), without tufting. There was some abortive tufting in a few birds given restricted diets and ascending salt levels, with or without some degree of glomerular enlargement. This was seen in birds with the highest heart weight–body weight ratios. In such instances the widened glomerular capillaries had at times a rigid, wire loop appearance, associated with some thickening of the basement membrane, but largely the result of pericapillary reticulosis.

Increased amounts of reticulum were deposited beneath and about the glomerular capillaries, as well as to some extent in the core itself, to a variable degree in all treated birds except those given 6 Gm. of food daily. It was most pronounced in groups 5, 7 and the few exceptions with abortive glomerular tufting in the calorically restricted groups. It was least pronounced in group 6.

Hypertrophy and hyperplasia of the visceral epithelium of the glomerulus occurred to about the same degree and under the same circumstances as reticulosis.

The slight arterial and arteriolar changes in groups 1 and 2 represented better preserved, i. e., less atrophic vessels rather than true hypertrophy. The thicker muscular fibers of the medial coat appeared

somewhat hydropic, and their larger nuclei were more vesicular. In all other groups except group 6 there were pronounced changes in these vessels. They were markedly tortuous, with thick-walled, frankly hypertrophied medial coats, out of proportion to the increased size of the kidney and not necessarily associated with glomerular hypertrophy (fig. 4). Neither intimal sclerosis, whether fibrosis or hyalinization, nor changes in the elastic lamellas, such as splitting, were observed. The arterial vessels in the experimental animals of group 6 were only slightly thicker and more tortuous than those of the controls, indicating here, again, the adaptation of previously hypertrophied vessels to the enlarging kidney as a result of growth, but that they were progressively more removed from the influence of the effects of sodium chloride.

There were certain other differences which merit comment. In rapidly growing birds with a high salt intake the following abnormalities are commonly found: hyaline droplet and fatty changes in the hypertrophied visceral epithelium of Bowman's capsule and mitotic activity of the hyperplastic epithelium; deposition of lipids in the subcapillary layers and tufts of the glomerulus; droplets of fat in tubular epithelium, particularly in that of regenerated sectors; the presence of red blood cell casts and associated with them regenerated sectors of tubules. Such changes were almost entirely lacking in treated animals of all other groups except in rare instances in which fatty deposition in glomeruli and tubular epithelium was more prominent. Glomerular crescents, occasionally noted in salt-treated actively growing animals, were sometimes observed in treated birds with a reduced caloric intake, particularly in the few with more pronounced glomerular changes and the largest hearts.

Tubular atrophy was evident in the kidneys of birds given restricted diets. This was less marked in treated than in control animals and involved the convoluted tubules to a greater extent than the distal collecting portions of the nephron.

In order to indicate that some advancement in age when the animal is first exposed to salt combined with relatively mild restriction of food intake will fail to elicit all the renal changes observed in young chicks with a high salt intake but fed ad libitum, the following experiment is recorded:

Six week old birds were maintained on an ascending scale of salt, from 2 per cent to 9.1 per cent, but fed 60 Gm. of food daily. There were corresponding controls with the same food intake but without salt. The experiment lasted forty-five days. The average heart weight–body weight ratio of the 7 controls was 0.53, while that of the 8 treated animals was 0.73. The average renal weight–heart weight ratio was 0.63 for the controls and 0.76 for the experimental birds. Microscopically, the experimental kidneys revealed marked hypertrophy and tortuosity of blood vessels. The glomeruli, however, were not nearly as large or as fully tufted as those of treated birds fed ad libitum.
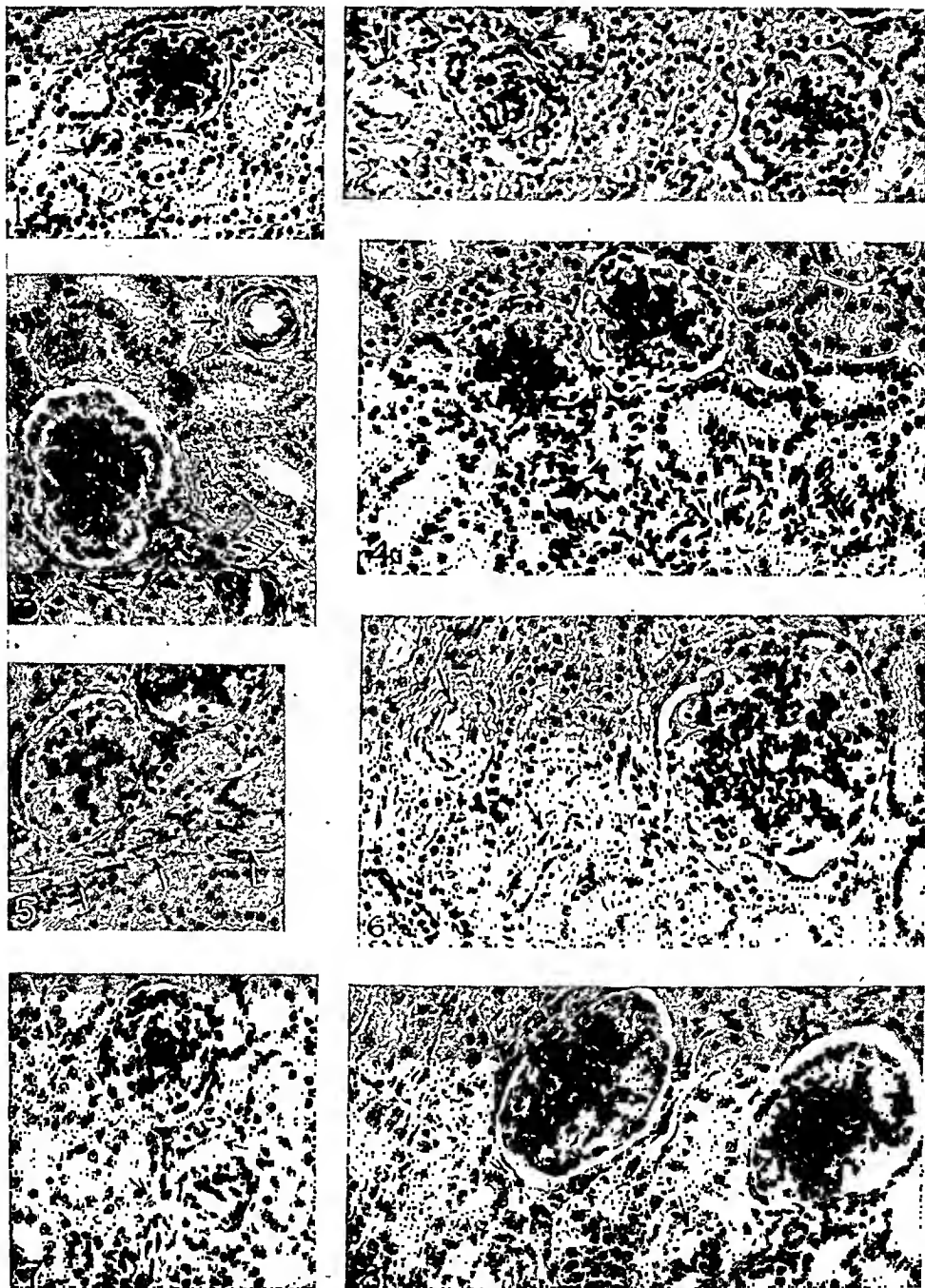
Photomicrographs of kidneys. Magnification, × 600. Arrows indicate arterial blood vessels.

Fig. 1.—Control chick aged 62 days. The bird had consumed 12 Gm. of food daily for thirty days.

Fig. 2.—Experimental chick aged 53 days. This bird had consumed 12 Gm. of food and ascending amounts of sodium chloride daily for thirty-one days. Note the visceral epithelial hypertrophy, the looped capillaries and the increase in reticulum of the glomerulus to the right. The artery to the left is more tortuous

COMMENT

It was the purpose of this study to determine whether the characteristic cardiorenal changes observed in young chicks given a 3 per cent salt diet and fed ad libitum could be reproduced under a variety of nutritional states with variations in the concentration and daily levels of salt. It seems clear from the data given that such changes are obtained in their entirety only when there is rapid and unhampered growth. Nutritionally induced disturbances of growth or the natural decline of the rate of growth prior to maturity or its cessation after maturity interfere with their full evolution. Provided certain daily levels of salt are reached, which on the average must exceed 0.3 Gm. per hundred grams of body weight, neither the concentration of salt in the food nor a progressive increase in the amount ingested daily would seem to be essential factors. The concentration of salt must not, however, be so high as to interfere with the animal's consumption of food. The amount of water ingested, to which we ascribed considerable importance in a previous communication, can in no way be separated in its effects on the heart and the kidneys from that of the high salt intake. The two must perforce be intimately linked if water intoxication is to be avoided. With levels of salt above 0.3 Gm. per hundred grams of body weight, water consumption will automatically exceed 50 cc. per hundred grams of live weight of bird daily.

Cardiac enlargement was a constant finding common to all groups with a high salt intake. The degree of hypertrophy appeared to be unrelated to the amount of salt ingested daily, provided this exceeded the basic limit, and to the growth and nutritional status of the animal,

---

and thicker walled than the control vessels in figure 1. This degree of glomerular change was exceptional for salt-treated birds on this diet.

Fig. 3.—Control chick aged 61 days. This bird was fed 23.5 Gm. of food daily for thirty-one days.

Fig. 4.—Experimental chick aged 70 days. The chick was given 23.5 Gm. of food and ascending amounts of sodium chloride daily for forty days. Note the absence of glomerular changes but the pronounced tortuosity and thickness of the preglomerular artery as compared with the control in figure 3.

Fig. 5.—Control chick aged 72 days. The bird was allowed to consume food ad libitum.

Fig. 6.—Experimental chick aged 72 days. This bird received food containing sodium chloride, 5 per cent, ad libitum for sixty-five days. Note the marked degree of glomerular hypertrophy with newly formed broad tufts or papillae. There is increased deposition of reticulum within the latter. There is also visceral epithelial hypertrophy. The tortuous hypertrophied blood vessels are clearly apparent.

Fig. 7.—Control bird aged 196 days, allowed food ad libitum.

Fig. 8.—Experimental bird aged 196 days. This bird had food ad libitum with ascending amounts of sodium chloride for thirty-eight days. It was an exception in presenting enlarged glomeruli of these dimensions. Note, however, their smooth character associated with increased size of the core and increase in number of nuclei but without tufting or papillary formation. The medial coats of the preglomerular arteries are evidently hypertrophied.

provided there was no profound loss in body weight. These hearts were 31 to 62 per cent heavier than their respective controls when weights were expressed per unit of body weight, and these values were highly significant statistically in all instances. When there was marked loss of body weight, 25 per cent or more over the experimental period, cardiac weight tended to be retained at the preexperimental level or to the extent of 16 to 18 per cent above that of similarly undernourished controls.

Since high salt intake is instrumental in elevating the mean blood pressure in birds, it may be pertinent to inquire whether cardiac hypertrophy was the result of this elevation. From our limited data, however, the increase in blood pressure was quite variable and seemed to be appreciable only when body weight was kept relatively stable in the young bird or when loss of weight was prevented in the adult animal. Since cardiac hypertrophy was a general phenomenon, it would seem unlikely that it was entirely due to hypertension. Other factors undoubtedly played an important role in its production.

The effect of high salt intake on the kidney was far more variable. Glomeruli, tubules and arterial blood vessels seemed to respond proportionately and as a unit only in chicks in which growth was unhampered. In all others, in which growth or nutrition was not in accord with this, these various elements responded independently if at all.

Glomerular hypertrophy was marked and constant only in rapidly growing birds. It failed to appear when loss of body weight was appreciable and was rarely pronounced and almost always symmetric in the other groups in which growth was either sharply restricted or inhibited or in well fed adults. The development of new glomerular tufts or papillae occurred only with unhampered growth. In view of these findings it is doubtful whether marked glomerular hypertrophy, particularly the new formation of tufts or papillae, can be regarded as a compensatory mechanism only. It is rather to be viewed as an exaggerated response to the stimulus of excess sodium chloride at a time when body growth is active.

Increase in tubular mass as represented by increase in renal weight was in most instances not significant statistically. Nevertheless, it seemed to follow that increase in renal weight varied directly with the increase in daily salt intake. It was, however, unrelated to glomerular or vascular changes.

Increase in tortuosity and medial hypertrophy of the interlobular arteries and their preglomerular arterioles in the inner third of the renal cortex was a constant finding. It varied in degree. It was most pronounced in salt-treated chicks fed ad libitum but was a promi-

nent feature in all other experimental groups except those with marked reduction of body weight. Here the integrity of the vessels was more apt to be maintained with respect to what they were prior to the experimental period, by contrast with the marked atrophy in the control animals. There was no strict correlation between these vascular changes and glomerular or tubular hypertrophy or hypertension. They seemed to be associated more closely with altered hemodynamics of the kidney.

SUMMARY AND CONCLUSIONS

Young chicks and older birds were divided into groups and fed either calorically restricted diets or food ad libitum. Within each group there were control and experimental animals. The latter were fed varying concentrations of sodium chloride with their food or, independent of the concentration, a fixed or an ascending amount of salt daily. The amount of sodium chloride ingested varied from 0.17 to 0.74 Gm. per hundred grams of body weight daily in different experimental groups and during different periods within certain of these groups. This did not include the amount of salt ordinarily present in the feed, which amounted to about 0.5 per cent.

In young chicks fed restricted diets the loss or gain in body weight in individual groups was respectively as follows: a loss of 30 to 36 per cent over two to three weeks; a loss of 23 to 27 per cent over two to four weeks; a loss of 4 to 7.5 per cent over three to four weeks; a gain of 25 to 50 per cent over four to six weeks. Young chicks allowed food ad libitum gained 85 to 117 per cent in body weight over a little more than a three week period.

The following conclusions are drawn from these experiments:

1. The daily load of sodium chloride apparently must exceed 0.3 Gm. per hundred grams of body weight if morphologic changes in heart and kidney are to take place. Water consumption, which under these circumstances will exceed 50 cc. per hundred grams of live weight of bird daily, cannot be dissociated in its effects from the salt load if water intoxication is to be avoided.

2. Cardiac hypertrophy is a constant finding in all experimental animals, independent of age, status of growth or amount of salt ingested above the basic limit. It is, however, largely relative and restricted to maintenance of the preexperimental weight of the heart when body weight has declined 25 per cent or more over the experimental period.

3. Renal hypertrophy, representing basically increase in tubular mass, is variable in its response to high salt intake. There are indications, however, that renal weight increases in direct proportion to the progressive increase of the daily load of sodium chloride.

4. Marked renal glomerular enlargement and the new formation of glomerular tufts or papillae occur only in experimental animals in which growth is rapid and unhampered. When growth is nutritionally restricted or when it is slowed or arrested as a result of natural aging processes, glomerular enlargement is at best limited, while newly formed tufts or papillae are practically never developed or are at most abortive.

5. Increased tortuosity and medial hypertrophy of renal arteries and preglomerular arterioles is a fairly constant finding in birds with a high salt intake. It is most markedly developed in animals in which growth is unhampered and is least pronounced in animals in which there has been profound loss in body weight.

6. The mean blood pressure is elevated in birds with a high salt intake, while the cardiac rate in young chicks is concomitantly diminished.

7. While the incidence of ascites is increased in calorically restricted animals with an elevated salt intake, hydropericardium is a frequent finding in all experimental animals, independent of the state of growth or nutrition.

8. Profuse intestinal hemorrhage occurs in birds on calorically restricted diets. It is as frequent in control as in experimental animals.

PIGMENT CHANGES IN EXPERIMENTAL WHOLE THICKNESS SKIN GRAFTS

DONALD E. BARKER, M.D.*

PHILADELPHIA

WHETHER pigment always invades the surrounding tissue after transplantation of skin has not been determined. On the one hand, Loeb,[1] Carnot and Deflandre[2] and Sale[3] showed that when 1 to 2 mm. pieces of black skin were transplanted to white areas the black pigment invaded the surrounding white area for a period of about ninety days. However, if similar white grafts were transplanted to pigmented areas, the pigment invaded the white graft, and later no trace of the graft was present. In 1932 Seevers and Spencer[4] reported that in the case of a pigmented rectangular graft with an area of about 2 sq. cm. there was no invasion of the surrounding area. Likewise, after a white graft of the same size was transplanted to a pigmented area, it was not invaded by the surrounding pigment during an observation period of nine months. In several cases the edges became slightly darker, but in no case was there actual pigmentary invasion. Seevers and Spencer also reported that the direction of the hair growth of the transplanted skin changed to conform to that of the surrounding skin.

Earlier work in this laboratory[5] with small grafts supported that of Loeb and others. It seemed possible, however, that migration of the pigment might not occur with larger grafts. For that reason I have carried out transplantations with large size grafts. This report concerns that work.

1. Loeb, L.: Arch. f. Entwcklngsmechn. d. Organ. 6:1, 1897.
2. Carnot, P., and Deflandre, C.: Compt. rend. Soc. de biol. 3:178, 1896.
3. Sale, L.: Arch. f. Entwcklngsmechn. d. Organ. 37:248, 1913.
4. Seevers, C. H., and Spencer, D. A.: Am. Naturalist 66:189, 1932.
5. Barker, D. E.: Arch. Path. 32:425, 1941.

EXPERIMENTAL PROCEDURE

Black and white guinea pigs weighing 8 to 13 ounces (227 to 368 5 Gm.) were' used. After clipping, the guinea pigs were shaved and washed, and the skin was sterilized with tincture of merthiolate. With a sterile cork borer 17 mm. in diameter, two circular areas of 2 27 sq. cm. were marked out, one in the black and the other in the white skin. The skin so outlined was excised and placed on a piece of gauze moistened with isotonic solution of sodium chloride. After hemostasis had been secured by pressure, the grafts were sutured into place, the black graft in the white area and vice versa. All of the grafts were placed at an angle of variance with the normal direction of the hair fibers The completed grafts were covered with petrolatum gauze and a moist sea sponge wrapped in gauze, and this dressing was held in place by an adhesive strap. The grafts were undisturbed for twelve days; then the dressings and the sutures were removed, and the progress was noted. In this way, black and white grafts of the same age and subject to the same conditions were observed Observations were made at intervals until the white grafts had disappeared completely.

RESULTS

In 11 of 50 guinea pigs takes of both the black and the white graft were obtained. These 11 animals are the only ones discussed here.
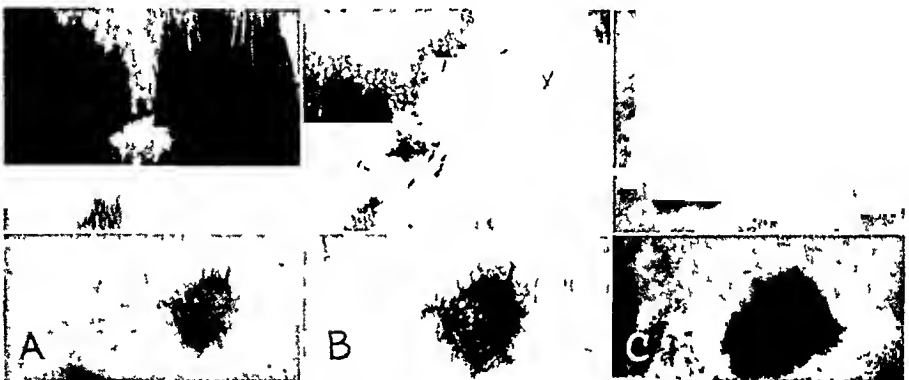
White and black skin grafts: *A,* forty days after transplantation. Note beginning invasion of the white graft. *B,* seventy days after transplantation. *C,* one hundred and two days after transplantation.

It was noted that during the first four weeks following transplantation the grafts shrank to about two thirds to one half of the original size. After the original shrinking stopped, the pigment of the black graft gradually invaded the surrounding white area. This extension of the black pigment continued for as long as ninety-three days. In direct contrast to this, in all 11 animals the white graft was gradually invaded by the surrounding black pigment until the graft was no longer visible. In 9 of the 11 guinea pigs this invasion of the white graft was completed within sixty to eighty days. However, in the remaining guinea pigs, 117 and 153, the process took one hundred and three and one hundred and twenty-nine days, respectively. These two retarded invasions are described in detail in the accompanying table.

The hair growth of the grafts was sparse, as many of the hair cells died during the transplantation. The hair grew out of the grafts in the original direction of growth. It was particularly interesting that in the case of the white grafts white hair continued to emerge from the grafted area for as long as five months, even though the original white graft had become completely pigmented.

COMMENT

The work presented here shows that when a large unpigmented skin graft is transplanted to a colored skin area the surrounding pigment gradually invades the graft. This result is in complete agreement with the earlier work of Loeb and others, who used smaller grafts, and is opposed to that of Seevers and Spencer. As Trotter and Dawson[6]

Detailed Presentation of Retarded Pigmentary Invasion of Two White Skin Grafts

Date of Observation	Black Graft, Mm.	White Graft, Mm.
Guinea Pig 117		
2/ 9 (day of operation)..............	17 × 17	17 × 17
3/18................................	12 × 15	12 × 8
3/27................................	15 × 18	10 × 7
4/ 5................................	17 × 18	9 × 6
4/17................................	20 × 22	6 × 4
4/27................................	21 × 23	3 × 2
5/10................................	22 × 23	0.5 × 0.5
5/19................................	22 × 23	0 × 0
Guinea Pig 153		
4/25 (day of operation)..............	17 × 17	17 × 17
5/19................................	5 × 5	10 × 7
5/23................................	5 × 5	10 × 7
6/ 5................................	10 × 9	4 × 7
6/18................................	15 × 12	1.5 × 2 and 1 × 1
6/24................................	15 × 12	0 × 0

have previously stated, the direction of the hair growth apparently remains constant, being unaffected by the movements and pressure of the surrounding hair growth.

SUMMARY

White skin grafted in place of colored skin is completely invaded by the surrounding pigment in sixty to one hundred and twenty-nine days. The hair color remains the same up to five months, even though the underlying grafted area becomes pigmented. The hair direction of the graft is not affected by external influences.

Graduate Hospital, Nineteenth and Lombard Streets.

6. Trotter, M., and Dawson, H. L.: Anat. Rec. **50**:193, 1931.

HOMOTRANSPLANTATION OF FETAL SKIN

DONALD E. BARKER, M.D.*

PHILADELPHIA

CLINICALLY, as early as 1884, Lucas [1] used skin from the prepuce of a newborn infant for homotransplantation with apparent success. This type of grafting was later revived by Eisenberg [2] and Ashley,[3] both of whom obtained results comparable with those of autotransplantation. Sabella [4] and Stern [5] reported success in using fetal membranes for the repair of burned or ulcerated surfaces. De Rotth [6] reported complete success in 8 cases of conjunctival defects repaired by the use of fetal membranes obtained at cesarean sections. Experimentally, Willis,[7] using skin from young rat embryos, reported excellent results with this tissue and suggested the use of materials taken from human embryos of the six to twelve week stage when sufficient skin is not available from the patient.

On the other hand, Lexer,[8] on the basis of his clinical observations, stated that although there was plainly an early enlargement of the epithelial islands following transplantation of skin of a fresh fetus, the entire flap was cast off in the third week. Coakley [9] reported that he and his co-workers were unable to get any better results with preputial skin than with adult skin. Experimentally, Skubisrewski [10] buried embryonic material subcutaneously in the hen and followed the result by repeated biopsy. He found that early multiplication and proliferation of the cells occurred but that this was later followed by regression and encapsulation of this material. Sartori [11] implanted embryonic material

1. Lucas, R C.: Lancet 2:586, 1884.
2. Eisenberg, I. C.: M. Rec. 95:514, 1919.
3. Ashley, F.: Ann. Surg. 106:252, 1937.
4. Sabella, N.: M. Rec. 83:478, 1913.
5. Stern, M.: J. A. M. A. 60:973, 1913.
6. de Rotth, A.: Arch. Ophth. 23:522, 1940.
7. Willis, R. A.: Australian & New Zealand J. Surg. 9:119, 1939.
8. Lexer, E.: Ann. Surg. 60:166, 1914.
9. Coakley, W. A.: Personal communication to the author.
10. Skubisrewski, L.: Compt. rend. Soc. de biol. 93:1398, 1925.
11. Sartori, C : Abstracted, J. A. M. A. 87:67, 1926.

in rabbits' eyes; he noted that, although there was early growth, after a certain stage all the implants succumbed to rapid involution and were completely resorbed.

It is apparent from these reports that the status of transplantation of fetal skin has not been definitely settled. An attempt has been made here to compare the results of homotransplantation of fetal and adult skin as accomplished by a method that yielded 80 to 90 per cent success in autotransplantation.

EXPERIMENTAL METHOD

Black and white guinea pigs weighing from 8 to 13 ounces (227 to 368.5 Gm.) were used as recipients. A 1 mm. square of black skin from each fetus and each adult was transplanted to a white rump area by the skin flap procedure described previously.[12] The fetal skin was obtained from fetuses 1 to 7 cm. in length (twenty-five days to term) immediately after cesarean section; pure black mothers were used. Pentobarbital sodium was the anesthetic. For the transplantation of adult skin, guinea pigs 4 to 5 months of age were used.

The term "initial takes" as used in this paper refers to the grafts which survived the early period following transplantation and were adherent and apparently well nourished when the flap was removed. In guinea pigs 19 and 126 the flap was accidentally disturbed earlier, but the grafts, even though exposed, progressed as described.

The survival time of the graft, given in days, refers to the period from the time of transplantation of the skin until the black graft was no longer visible.

RESULTS

For convenience the experiments have been placed in the following groups: group 1 (fetuses 1 cm. in length), 16 grafts; group 2 (2.5 cm. fetuses), 18 grafts; group 3 (4 cm. fetuses), 58 grafts; group 4 (4.5 cm. fetuses), 52 grafts; group 5 (5 cm. fetuses), 22 grafts; group 6 (6 cm. fetuses), 24 grafts; group 7 (adult tissue), 66 grafts. In the 16 experiments in group 1 (1 cm. fetuses) there were no takes. This was true also of the grafts in group 2 (2.5 cm. fetuses).

Eight of the 58 grafts in group 3 (4 cm. fetuses) showed initial takes. In this group most of the grafts remained the same size until their disappearance thirty-two to fifty-nine days after operation. An exception to this was guinea pig 84, whose graft increased from its original size of 0.1 by 0.1 mm. to 3 by 2 mm. by the thirtieth day but then seemed to melt away and was gone two days later.

Four of the 52 grafts from group 4 (4.5 cm. fetuses) were successful. Of these the graft on guinea pig 19 had increased to 4 by 2 mm. nineteen days after operation; it then gradually became smaller and disappeared fifty days after transplantation. The graft on guinea pig 126 had increased to 5 by 1.5 mm. nineteen days after operation, but disappeared nine days later.

Six of the 22 grafts in group 5 (5 cm. fetuses) were initial takes and grew for a short period. The graft on guinea pig 11 had increased to 10 by 3 mm. after operation, but gradually began to regress, and disappeared in twenty-seven days.

Only 1 of the 24 grafts in group 6 (6 cm. fetuses) showed an initial take. In this animal the graft remained 2 by 1 mm. for forty-five days after transplanta-

---

12. Barker, D. E.: Arch. Path. **32**:425, 1941.

tion, then began to fade, and was no longer visible sixty-five days after the operation.

Of the adult series (group 7) of 66 experiments, 14 (21 per cent) were successful at first. In 12 of these the graft remained the same size for a period of twenty-one to twenty-four days and then gradually became smaller until it disappeared. The graft on guinea pig 60, however, had increased to 3 by 2 mm. by twenty-seven days after transplantation. Seven days later it was noted that the graft was beginning to fade, and it was no longer visible forty-five days after transplantation. The graft on guinea pig 56 increased to 3 by 1 mm. and lasted for forty-one days after operation.

COMMENT

In this series of experiments in which skin grafts from guinea pig fetuses of 1 to 7 cm. in length were used, apparently the younger is not as well prepared to withstand transplantation as is the older fetal skin (see table). The percentage of primary takes when the older fetal tissues were used is about comparable with that recorded when adult tissues were used. The main difference between the use of older fetal

Transplantation of Adult and Fetal Skin

Group	Length of Fetus, Cm.	Experiments	Initial Takes	Percentage of Initial Takes	Percentage of Permanent Takes
Fetal tissue 1...................	1	16	0	0	0
2...................	2.5	18	0	0	0
3...................	4	58	8	13	0
4...................	4.5	52	4	7.7	0
5...................	5	22	6	27	0
6...................	6	24	1	4	0
Adult tissue...................	..	66	14	21	0

tissues and that of adult tissues is that with the fetal tissues there are early proliferation and multiplication of the cells with enlargement of the graft, while, on the whole, the adult grafts do not increase in size. The fetal grafts usually disappear by a slow decrease in size. Loeb [13] showed that with homotransplantation of adult tissues there is gradually a decrease in the number of mitotic figures present following transplantation until the seventh day, when all have apparently gone. In the use of fetal tissues this mitosis apparently persists up to about the twenty-fifth day, when regression sets in. The results given here are in agreement with the works of Lexer,[8] Skubisrewski [10] and Sartori.[11]

CONCLUSIONS

In the guinea pig the use of fetal skin tissues for permanent transplantation is apparently no more successful than that of adult tissues.

The early proliferation of the fetal skin is of short duration and is followed by either a rapid melting away of the graft or a slow regression.

Graduate Hospital, Nineteenth and Lombard Streets.

13. Loeb, L.: Arch. f. Entwcklngsmechn. d. Org. **27**:73 (Jan.) 1909.

COMMON TRUNK OF PULMONARY VEINS TRIBUTARY TO THE PORTAL VEIN, WITH A MULTIPLE CARDIAC ANOMALY

MILLINGTON O. YOUNG, M.D.

NEW HAVEN, CONN.

A PULMONARY venous system draining in whole or in part into the portal vein is rare. In 1942 Brody[1] collected 102 cases in which part or all of the pulmonary venous system drained into the right side of the heart or its tributaries and described 4 additional cases. Two years later, Hughes and Rumore[2] reported 2 instances and collected 7 cases, not mentioned by Brody, from the literature. However, they did not include the case reported by Conn and others,[3] who in turn referred to the cases of Barge and van Oijen[4] and Goltman and Stern,[5] which are not tabulated elsewhere. In a recent paper on the surgical significance of anomalies of the pulmonary veins, Brantigan[6] referred to 7 cases more recently reported and added 2 cases (in one the anomaly was observed by him at operation, and in the other it was visualized through a thoracoscope). Among the 127 cases there were only 5 (all cited by Brody) in which all or part of the pulmonary venous system drained into the portal vein. The only case of association of a cardiac anomaly and a pulmonary venous system draining completely into the portal vein was reported by Arnold,[7] in 1868. An additional case of this type is recorded in the present paper. The anomalies occurred in a stillborn infant and were associated with additional developmental defects.

REPORT OF A CASE

A premature white infant was born dead during the estimated sixth month of pregnancy. The onset of labor followed a long bus ride taken by the mother. The

1. Brody, H.: Arch. Path. **33**:221, 1942.

2. Hughes, C. W., and Rumore, P. C.: Arch. Path. **37**:364, 1944.

3. Conn, L. C.; Calder, J.; MacGregor, J. W., and Shaner, R. F.: Anat. Rec. **83**:335, 1942.

4. Barge, J. A., and van Oijen: Ztschr. f. Anat. u. Entwcklngsgesch. **98**:760, 1932; cited by Conn and others.[3]

5. Goltman, D. W., and Stern, N. S.: Am. Heart J. **18**:176, 1939.

6. Brantigan, O. C.: Surg., Gynec. & Obst. **84**:653, 1947.

7. Arnold, J.: Virchows Arch. f. path. Anat. **42**:449, 1868.

presentation was a double footling. The mother, aged 25 years, had 3 children, who were delivered uneventfully at term. During the third pregnancy, two years previously, a diagnosis of syphilis was made, and treatment was carried on during the pregnancy and subsequently. On this admission, the Mazzini test of the blood was negative; a test for the Rh factor was positive.

At necropsy the fetus was well developed and well proportioned; it was 31 cm. long and weighed 605 Gm. There were no anomalies of the mouth. The neck, chest, abdomen, external genitalia and back were as usual. The upper and lower extremities were proportionate and symmetric.

Ten centimeters of moist cord was attached to the umbilicus. The umbilical vein and remains of the urachus appeared as usual. The right umbilical artery was absent; the left measured 0.3 cm. in diameter. The cecum was mobile and in the right upper quadrant of the abdomen. A 2 cm. segment of colon, composed of the distal ascending and proximal transverse portions, did not have a mesentery. The spleen was absent. The liver was located on the right, with the lobes of the usual proportions. The stomach and the pancreas were in the right upper quadrant of the abdomen. The first portion of the duodenum and the head of the pancreas curved posteriorly on themselves. The distal portions of the duodenum were in their usual locations.

There were no ossification centers in the sternum. The costochondral junctions were straight. The thymus gland was bilobed and measured 1.3 by 0.7 by 0.5 cm.; from the upper margin of each lobe a finger-like projection extended into the root of the neck for a distance of 0.8 cm. The pleural cavities contained no excess fluid; their surfaces were smooth and glistening.

The pericardial cavity contained no excess fluid; its surfaces were smooth and glistening. The heart measured 2.5 cm. from base to apex and 2.5 cm. across the base. The apex pointed slightly to the right and was made up entirely of the left ventricle. The aorta arose from the diminutive right ventricle (fig. 1A). The pulmonary artery seemed to arise from the left ventricle. There was only one atrium. The single atrioventricular orifice had a bicuspid valve and opened into the left ventricle. No pulmonary orifice was present. An interventricular septal defect, 0.7 cm. in diameter, was present in the superior portion of the septum (fig. 1 B). The atrium did not communicate with the right ventricle. The aortic orifice was 0.9 cm. in circumference; its valves were delicate. The orifices of two coronary arteries were present in the left sinus; the orifice of one, in the right.

The innominate and left common carotid arteries arose from the arch of the aorta as a single trunk. The ductus arteriosus was patent, easily admitting a probe. The descending portion of the thoracic aorta and the abdominal aorta were on the left.

The tributaries of the superior vena cava were as usual, and the vein itself entered the right side of the atrium. Inferiorly the atrium received two venous trunks: a right hepatic vein on the right and a venous trunk continuous with the inferior vena cava on the left (fig. 2). A communicating branch connected the two trunks just before they entered into the atrium. The inferior vena cava was located on the right, inferior to the renal veins. Superior to the renal veins there was a single venous trunk on the left, formed by the persistence of the prerenal segment of the left subcardinal vein and the proximal portion of the left vitelline vein. Before entering the atrium, this vein received a left hepatic vein and the ductus venosus.

The left and right pulmonary veins united in the midline, posterior to the heart, and the single trunk thus formed descended to pass through the diaphragm, anterior
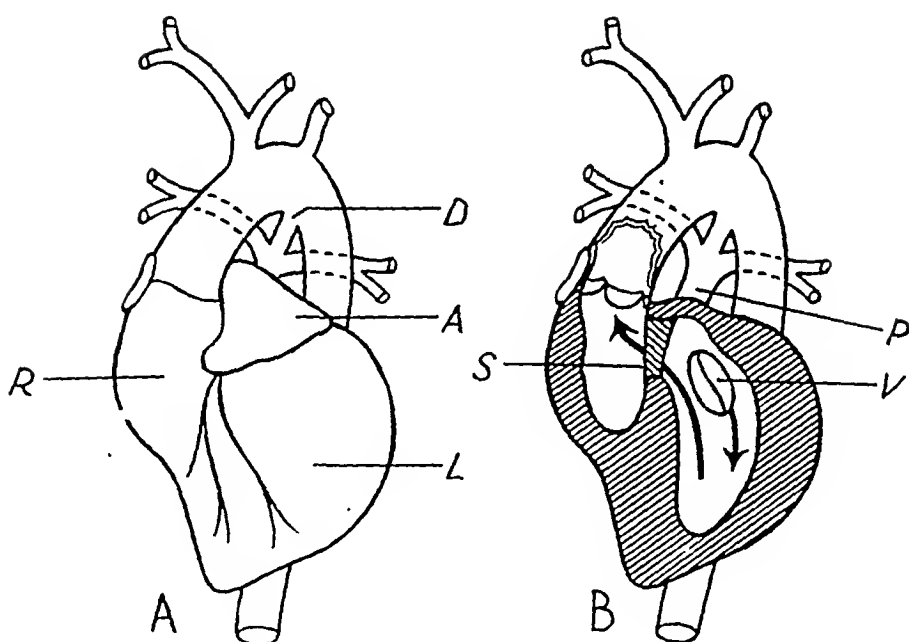
Fig. 1.—*A*, anterior view of the heart: *D*, patent ductus arteriosus; *A*, single atrium; *R*, right ventricle; *L*, left ventricle.

B, anterior view of heart, exposing the chambers and orifices: *P*, pulmonary artery with atretic orifice; *V*, single atrioventricular valve; *S*, interventricular septal defect.
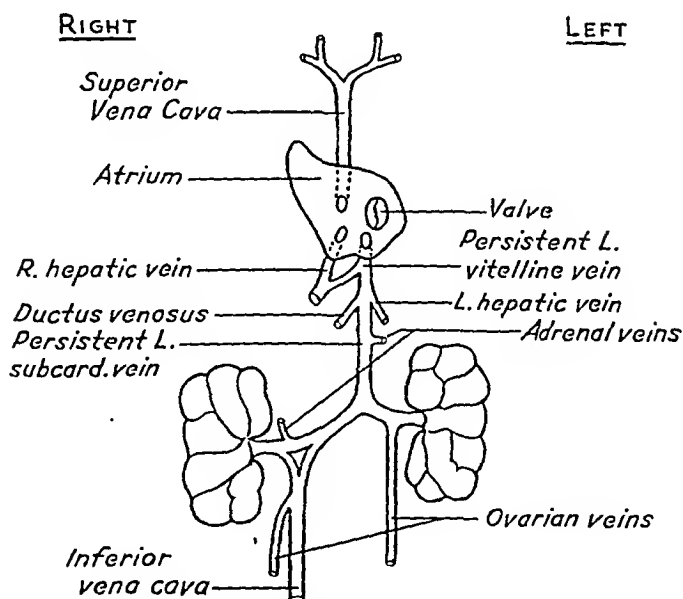

Fig. 2.—The atrium and its tributaries. The anomalous position of the vessels inferior to the atrium is shown in their relation to the kidneys.

to the esophagus (fig. 3). The common trunk received a tributary vein from the lesser curvature of the stomach (coronary vein) before uniting with a trunk formed by the union of the superior and inferior mesenteric veins to form the portal vein. The splenic vein was absent.

The lungs were not air containing. The left had three ·lobes; the right had an incompletely formed ·fourth lobe composed of the superior portion of the inferior lobe. The liver, the gallbladder and the extrahepatic biliary ducts were of usual size and appearance. The adrenal glands were also of usual size. The right adrenal vein emptied into· the right renal vein; the left, into the· persistent prerenal segment of the left subcardinal vein. The kidneys were lobulated and of proportionate size. The upper pole of the left kidney was slightly higher than that of the right, as usual. The vagina, uterus, fallopian tubes and ovaries were of proportionate size.
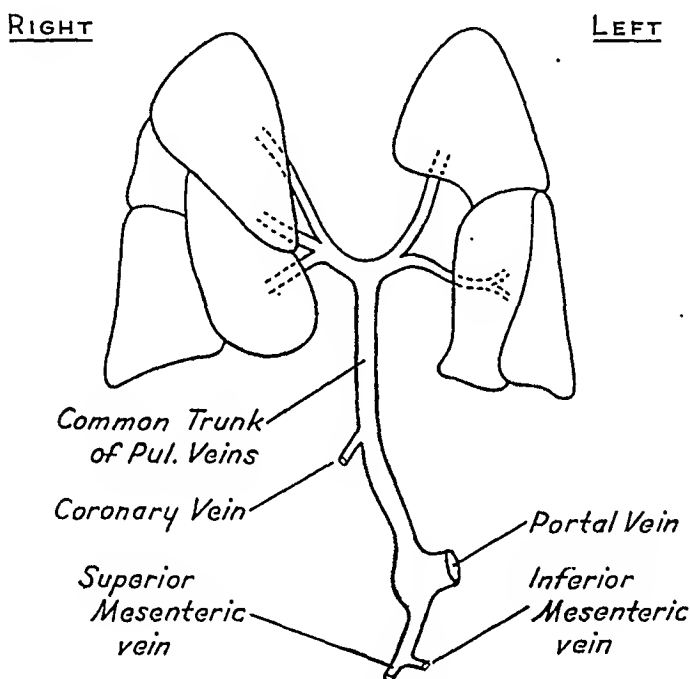
Fig. 3.—The pulmonary venous system. The common trunk formed by the union of the pulmonary veins receives the coronary vein, inferior to the diaphragm, before uniting with a common trunk of the mesenteric veins to form the portal vein. The first portion of the portal vein was directed to the left as indicated, although the liver was on the right side as usual. · ·

In the cranial cavity, the falx cerebri and the tentorium cerebelli were intact. No anomalies or changes were noted about the brain, its· vessels and supporting structures.

The findings are summarized in the following anatomic diagnosis: prematurity; atelectasis of the· lungs, fetal; trilocular heart with two ventricles, absence of the right atrioventricular orifice, interventricular septal defect, transposition of the arterial trunks with atresia of the pulmonary orifice and patency of the ductus arteriosus; common trunk of the pulmonary veins tributary to the portal vein; right hepatic vein entering into the atrium; persistence of the proximal left vitelline vein and of the prerenal segment of the left subcardinal vein with atresia of the prerenal segment of the right subcardinal vein; atresia of the right umbilical artery; situs inversus of the stomach and the pancreas with aplasia of the spleen.

COMMENT

In an early stage of embryonic development, an anastomosing network of capillaries comprises the circulatory system.[8] As the embryo enlarges, parts of this system differentiate to form the heart, the arteries and the veins. Concomitantly, certain of the channels enlarge and others disappear as the various vascular systems develop. At one stage the umbilical, vitelline, splanchnic and cardinal venous systems converge in the septum transversum and anastomose with one another. Most of the communicating vessels disappear. Occasionally, some remain.

Persistence of one or more of the primitive connections between the pulmonary and splanchnic or vitelline systems accounts for one group of anomalies observed. In such an instance the fully developed pulmonary venous system partially drains into any of the tributaries of the right atrium derived from the cardinal system or into the portal vein, which is derived entirely from the vitelline system. All or part of the pulmonary veins may be diverted into the right atrium when the heart develops abnormally or when the primitive pulmonary vein connects at an unusual site. If the primitive pulmonary vein becomes atretic or fails to connect with the heart, one or more of the channels communicating with the splanchnic or the vitelline plexuses remain patent. Thus, all the blood returning from the lungs would empty into one or more of the tributaries of the right atrium or into the portal vein, as occurred in the case herein presented.

The circulatory system in this case was in effect that of a two-chambered heart (fig. 4). All venous blood returned to the single atrium. From there it reached the left ventricle, which did not communicate with the pulmonary artery. The left ventricle pumped blood through the interventricular septal defect and the right ventricle into the aorta. The patent ductus arteriosus allowed blood to enter the pulmonary arteries.

The initial defect of development was probably the failure of the primitive pulmonary venous system to drain into the heart. Since, under these circumstances, it was impossible to segregate the oxygenated and unoxygenated blood, this initial defect might have been the cause of the failure of the heart to develop further.

This type of circulatory system is apparently compatible with life only on a limited basis. In the 3 previous cases in which the total pulmonary venous system drained into the portal vein, the duration of life was fifteen weeks,[7] fifteen days [9] and three months,[10] respectively. In

8. Evans, H. M., in Keibel, F., and Mall, F. P.: Manual of Human Embryology, Philadelphia, J. B. Lippincott Company, 1912, vol. 2, p. 580.

9. Ghon, A.: Beitr. z. path. Anat. u. z. allg. Path. 62:175, 1916; cited by Brody.[1]

10. Munck: Acta path. et microbiol. Scandinav. 10:321, 1933; cited by Brody.[1]

the case herein reported, the infant might have lived as long as the ductus arteriosus remained sufficiently patent to allow adequate circulation to the lungs.

The cardiac anomaly in Arnold's[7] case was in certain respects similar to the one reported here. The heart was in effect a two-chambered system, as a wide communication was present between the atriums and there was only a single ventricle. There was also atresia of the pulmonary orifice with patency of the ductus arteriosus. In addition, the spleen was absent.
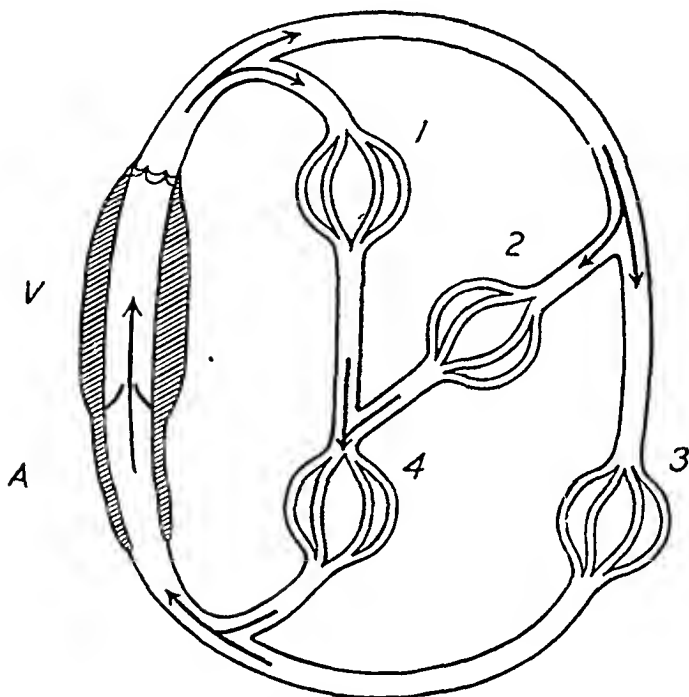
Fig. 4.—Diagram of the circulatory system: *A*, atrium; *V*, ventricle; *1*, pulmonary circulation; *2*, mesenteric circulation; *3*, systemic circulation; *4*, portal circulation.

Another unusual finding deserves comment. It is believed that the leftward rotation of the stomach is due to the mechanical influence of the rapid growth of the liver on the right side. In my case both the stomach and the liver were on the right side. It seems likely that the presence of the common trunk of the pulmonary veins inferior to the diaphragm may have interfered with the usual rotation of the stomach.

SUMMARY

A pulmonary venous system draining completely into the portal system was observed in a premature, stillborn girl, associated with a multiple cardiac anomaly and other developmental defects. This case

is believed to be the sixth reported instance of a pulmonary venous system draining in whole or in part into the portal vein and the fourth case in which such anomalous drainage was complete. It is apparently the second instance in which complete drainage was associated with a cardiac anomaly and the first case in which it was associated with this particular cardiac anomaly.

Department of Surgery, Yale University School of Medicine.

STUDIES IN DYSTROPHIA MYOTONICA

VII. Autopsy Observations in Five Cases

W. C. BLACK, M.D.

AND

A. RAVIN, M.D.

DENVER

DURING the course of investigations of a group of patients with dystrophia myotonica at the Colorado General Hospital, 3 of the patients died, and autopsies were made. In comparing the sections from these autopsies with sections from autopsies made in 2 cases in which primary muscular atrophy was diagnosed, one of which was reported by Moleen, Johnson and Dixon [1] in 1932, we were struck by the similarity. A review of the histories obtained in these 2 cases convinced us that the patients had dystrophia myotonica instead of primary muscular atrophy. The pathologic changes found in these 5 patients are presented in this report. The heredity,[2] clinical features [3] and psychiatric aspects [4] of dystrophia myotonica have been discussed in previous articles of this series. Experimental investigations of myotonia,[5] creatine and creatinine excretion [6] and sugar tolerance [7] have also been reported.

REPORT OF CASES

CASE 1.—F. B., a white man aged 44 years, was first seen in April 1938, complaining of weakness of the hands and legs of many years' duration and recent hemoptysis.

The patient's father had died at about 75 years of age in a state hospital to which he had been committed with a diagnosis of senile dementia; his vision had been poor for a number of years before his death. The patient's mother was alive at 81 years, but mildly diabetic. (She died shortly after the patient's death.) The

1. Moleen, G. A.; Johnson, W. C., and Dixon, H. H.: Arch. Neurol. & Psychiat. **27**:645, 1932.

2. Ravin, A., and Waring, J. J.: Am. J. M. Sc. **197**:593, 1939.

3. Waring, J. J.; Ravin, A., and Walker, C. E.: Arch. Int. Med. **65**:763, 1940.

4. Billings, E. G., and Ravin, A.: Am. J. Psychiat. **97**:1116, 1941.

5. Ravin, A.: Arch. Neurol. & Psychiat. **43**:649, 1940; Medicine **18**:443, 1939. Ravin, A., and Waring, J. J.: Ann. Int. Med. **13**:1174, 1940.

6. Lewis, R. C., Jr.; Ravin, A., and Lewis, R. C.: J. Lab. & Clin. Med. **26**:990 1941.

7. Rymer, M. R., and Ravin, A.: J. Lab. & Clin. Med. **26**:1506, 1941.

patient was the ninth of thirteen children. An older sister died in her fifties of cerebral embolism and bronchopneumonia. She was apparently simple minded, allegedly as a result of an injury to the head in childhood. Another sister, aged 56, was mildly diabetic. One sister died at about 44 years of age of "heart trouble." She was ill the last twenty years of her life and had frequently said that her hands stuck to things. Her husband said that often, on the first bite, her jaw would stay clamped for a while and that her jaw dislocated easily. An older brother, about 51 years old, was thin, walked with a "steppage" gait and was said by one of his brothers to look just like the patient; he wore glasses and had poor vision. One sister died at 16 months of age of diphtheria. Two younger brothers and two younger sisters apparently were normal.

The past history revealed that the patient had had mumps with no associated orchitis. He had typhoid fever at the age of 14 and pneumonia at the age of 22. At the age of 30 he was in the hospital with a pain in the right side which was attributed to a ureteral stricture. He had gonorrhea at the age of 22 and again at that of 33; following the second attack he had some joint pains and swelling. He went through the eighth grade and then did tire repair work and odd jobs. He married at the age of 22 and was divorced twelve years later. His wife had no children, although he said she was pregnant on several occasions and induced abortions.

At the age of 16 or 17 the patient first noticed that when he gripped an object strongly he had difficulty releasing it. At 22 to 23 years of age he realized that his grip was not as strong as that of his fellow workers. The difficulty in relaxing his grasp and the loss of strength in his grip gradually progressed. The strength in his arms and shoulders had also decreased. Two years before coming to the clinic he had been told that he walked as though he had a "wooden leg or was paralyzed." Since that time he had been conscious of increasing difficulty in walking. If while standing he became too interested in what he was doing, he· was apt to lose his balance and have to take a step to regain it. During the last year he had noted stiffness of his ankles on starting to walk. As far back as he could remember his hands and feet had been cold. In the last six months his voice had become lower in pitch, less clear and somewhat husky. Occasionally he had difficulty in drinking water from a fountain. Print blurred when he read, and his eyes frequently burned and watered.

About six weeks before being examined the patient began to have some pain in the right costal region, which was worse on deep inspiration. The pain was associated with some cough and expectoration of a little mucoid material. After a week the pain went to his left side, and he coughed up some bloody sputum. The pain lasted about three weeks. He believed that he had had some fever with the onset of the pain. He was not short of breath and had noticed no edema.

The patient was a tall, well nourished, middle-aged white man. He was intelligent and cooperative. His voice was definitely nasal and his gait of the "steppage" type. He was bald with a graying rim of hair.

There was slight marginal blepharitis and chronic conjunctivitis. Slit lamp examination after use of homatropine hydrobromide showed many fine opacities, varying in size from that of dust particles to almost a millimeter in diameter, scattered throughout the cortex of the lens and extending well into the central area. They were grayish white but refractile and appeared blue and blue-green at times. The fetal nucleus was not involved. Vision with glasses was 20/20 in both eyes.

Most of the teeth were present, although in poor condition. The thyroid gland was small but firm. The lungs were normal to percussion and auscultation. The heart was normal in size. The heart sounds were somewhat faint but normal in

character. The aortic second and the pulmonic second sound were of about equal intensity. No murmurs were heard. Peripheral sclerosis was not evident. The blood pressure was 96 systolic and 64 diastolic in the recumbent position and 104 systolic and 72 diastolic in the sitting position. The systolic pressure fell about 10 mm. with deep inspiration.

Abdominal examination gave negative results. Both testes were possibly somewhat softer and smaller than normal.

Examination of the neuromuscular system showed the following variations from normal: The temporal muscles were markedly atrophied and the masseter muscles somewhat atrophied. The periorbital and perioral muscles were weak. The sternocleidomastoid muscles were almost completely atrophied, and when the patient rose from a reclining position his head fell backward and had to be supported by his hand. The muscles of the supraclavicular fossae and the deltoid muscles were somewhat atrophied. The biceps and especially the triceps muscle on each side were weak and somewhat atrophied. Both the flexor and the extensor muscles in the forearm were weak and atrophic. The muscles of the thenar and hypothenar eminences were only slightly involved but the interossei muscles were definitely atrophied. Flexion and extension of the thigh were fairly good, as was extension of the knees. Flexion of the knees was weakened. Plantar flexion of the feet was fair on the left and weak on the right. Dorsiflexion of the feet was markedly decreased.

The patellar and the abdominal reflexes were present and normal. The biceps and achilles reflexes could not be obtained. Chvostek's sign was positive. Trousseau's sign was negative.

Voluntary myotonia (persistence of the contraction of a muscle after cessation of voluntary effort at contraction) was evident in the movements of the fingers, thumbs and ankle. When the patient flexed his fingers, they could be extended only very slowly in spite of marked effort. The determined effort to overcome the persistent contraction in flexor muscles resulted in a rather characteristic distortion of the upper extremities: The elbows and the wrists were flexed to facilitate the extension of the fingers, and the wrist was gradually extended as the fingers slowly became extended—the small finger usually being the first to be extended completely and the index finger the last. The second time that the flexor muscles were contracted, extension occurred more rapidly, although still more slowly than normal. With each repetition of the movement the extension became more rapid until it occurred with normal rapidity. After a period of rest the difficulty returned.

The mechanical irritability of the muscles was generally increased; that is, they contracted more readily when struck with a percussion hammer than normal muscles. Many of the muscles, furthermore, on contracting remained contracted for many seconds (mechanical myotonia), forming a furrow or dimple, and then slowly relaxed. This was seen in the tongue, in the chin, in the deltoids, biceps and triceps muscles on each side, the flexor and extensor muscles of the forearm, the muscles of the thenar and hypothenar eminences, the quadriceps muscle on each side, and the left gastrocnemius muscle.

The Wassermann and Eagle tests of the blood were normal. The spinal fluid was normal. Roentgenologic examination of the chest on April 21 revealed no abnormalities of the heart or the lungs. The gastrointestinal tract was normal except for marked spasticity and stasis in the colon. On April 25 the blood serum calcium was 9.3 mg. and the serum inorganic phosphorus 2.8 mg. per hundred cubic centimeters. The blood cholesterol was 148 mg. per hundred cubic centimeters. The basal metabolic rate was —26 per cent. The electrocardiogram was

normal except for a somewhat high take-off of the S-T segment in lead I (1 mm.) and in lead II (1 mm.).

Course.—Injections of 25 mg. of testosterone propionate were started at the rate of two a week. On May 5 the patient again began to have a severe pain in the right costal region and coughed up some bloody sputum. He was admitted to the hospital on May 6 with these complaints and a temperature of 100 F. At about 4 a. m. the following day the patient suddenly became cyanotic and comatose. Respirations dropped to 3 or 4 per minute, and the trachea and lungs were full of moisture. In an oxygen tent and with stimulants, the patient improved. A roentgenogram of the chest revealed a homogeneous density of the right lower lobe and some increase of density of the left lower lobe. Sputum culture revealed the presence of Pneumococcus, type III. The patient was then treated with sulfanilamide, but the course was downhill and he died on May 10. The observations at autopsy are recorded in a subsequent section.

The clinical picture of this patient to the time of his last illness is typical of dystrophia myotonica. The features of the disease which he showed are: (1) the heredity characteristic of patients with the disease, (2) myotonia, (3) muscle atrophy, (4) cataracts, (5) baldness and a (6) low basal metabolic rate. The family history is in many ways characteristic of that usually obtained, in that several siblings were involved and the parents were stated not to have had the disease. In a previous article of this series [2] it was concluded that the disease is probably transmitted as a dominant characteristic but that it tends to become more severe and to set in at an earlier age in each succeeding generation. In the affected parent of this patient, the onset of the disease was probably late in life, and those changes which occurred before death were not of sufficient degree to be distinguished from the expected senile changes. This, we believe, is the reason why the disease, although transmitted as a dominant character, is frequently not recognized in the parents.

The distribution of the myotonia—that is, in the hand grasps and in the legs—is characteristic of the disease. Occasionally, as in the patient's sister, the myotonia is also seen in some of the muscles of mastication; rarely it may be widespread. The presence of myotonia is of great value in distinguishing these patients from patients with other types of muscular atrophy.

The tendency of the muscle atrophy of dystrophia myotonica to involve certain muscles early is well shown in this man. The regions usually involved include the muscles of the forearm, the sternocleidomastoid muscles, the dorsiflexors of the feet, the facial muscles and the quadriceps muscles. As the disease progresses, the atrophy becomes more general. The atrophy and the weakness are the main causes of disability and usually the motive of the patients' seeking medical advice.

Presenile cataract, if looked for with a slit lamp, can be found in most patients with advanced muscle atrophy. Early stages of the cataract may at times be present before the muscle changes.

Although myotonia, muscle atrophy and cataract are the triad by which the disease should be remembered, other dystrophic changes and endocrine changes occur frequently. The baldness, the low basal metabolic rate and the hypotension are evidences in this patient of some of these changes. Testicular atrophy is also commonly seen. Menstrual irregularities, infertility and enlargement of the thyroid gland may also be seen.

CASE 2.—O. M., a 43 year old man, first came to the clinic in December 1935, complaining of poor vision. Two sisters, a brother and a nephew were also considered as having dystrophia myotonica. His father was operated on for cataract at about 60 years of age; a sister of his father was operated on for cataract at 65 years of age; a half-sister of his mother was thought to have had "locomotor ataxia," because she suffered from progressive inability to use her lower extremities from about the age of 30 years to her death at 64 years.

The patient was born in Illinois in 1895. He finished the eighth grade at the age of 16 years and then worked as a farmer and as a carpenter. He enlisted in the Army in 1917 and was discharged in 1919. He had measles, mumps and whooping cough in childhood and pneumonia and pleurisy in 1930. He was married in 1924 and had no children.

He wore glasses from the age of 12. At the age of 30 he was told that he had incipient cataracts. A few years later his vision began to fail rapidly, and at 40 years of age he was almost blind. Examination at that time revealed almost mature cataracts of both eyes. Slit slamp examination showed an almost complete opacity of each lens, with highly refractile globular opacities, which cast bluish reflections. In January 1936 a cataract was removed from the left eye, and in February 1936 one was removed from the right eye. For many years he had had some irritation of his eyelids and a slight discharge from his eyes.

In 1922, at the age of 27, he noticed that he had a poor grip, and in 1928 he noticed that if he grasped an object he could not immediately release it. The weakness of his hand grasp gradually increased; the stiffness did not change much. On beginning movement after rest the muscles of the lower extremities felt stiff. The stiffness in his hands and legs was worse in cold weather.

For twelve to fourteen years before being seen the patient had been very intolerant to cold, and his hands and feet easily became cold. During the same time he lost both strength and energy. In October 1933 he was found to have a basal metabolic rate of —37 per cent. Thyroid U. S. P. was administered, and the basal metabolic rate increased to normal with a dose of 10 grains (0.65 Gm.) one day alternated with 5 grains (0.32 Gm.) the next day. With this treatment the patient's sluggishness and malaise were improved. When seen in December 1935 he was still taking thyroid but did not think he was greatly benefited. He felt that there had been no change in the stiffness of his hands.

Examination in January 1938 revealed a well nourished, rather tall, fairly well developed white man of 43 years of age, with an expressionless "hatchet facies." He talked rather slowly. The gait was slightly but definitely of the "steppage" type, with some "slapping." The lens of each eye had been removed. The thyroid gland was easily palpable, being enlarged about one and a half times. The lungs and the abdomen were normal. The testicles were somewhat smaller and softer than normal.

A roentgenogram revealed the heart and the aorta to be of normal size and shape. The heart sounds were faint. The blood pressure was 100 systolic and 68

diastolic, and the pulse rate was 56 per minute. The peripheral vessels showed little if any evidence of sclerosis.

Examination of the neuromuscular system revealed that the patient had the typical myopathic facies as a result of atrophy and weakness of the muscles of expression. The temporal muscles were moderately atrophied, but the masseter muscles appeared in good condition. The sternocleidomastoid muscles were markedly atrophied. The muscles of the forearm were moderately atrophied. The hand grasp was weak, more so on the right, although the patient was right handed. The extensor muscles of the forearm appeared more affected than the flexors. Flexion of the thigh and leg was somewhat weakened. Dorsiflexion of the feet was weak, but definite atrophy of the dorsiflexor muscles was questionable. Active myotonia was present to a marked degree in the hand grasp and to a much less degree in the movements of the toes and ankles. The mechanical irritability of the muscles was increased, and mechanical myotonia was evident in the muscles of the chin, the extensor muscles of the wrist and fingers, the tongue, the deltoid muscles and the muscles of the thenar and hypothenar eminences.

The Wassermann test and the Eagle flocculation test of the blood were negative. The urine was normal. The blood counts showed no persisting abnormality. A roentgenogram of the skull in January 1936 showed the sella turcica normal and the pineal body, partially calcified, in normal position. The basal metabolic rate on Oct. 15, 1933, was —37 per cent, and the cholesterol of the blood at the same time was 180 mg. per hundred cubic centimeters. While the patient was taking thyroid, the basal metabolic rate varied from —26 to plus 8 per cent. In December 1935 three determinations were made of the calcium content of the blood, showing 11.0, 10.1 and 11.2 mg. per hundred cubic· centimeters; the associated phosphorus values were 3.8, 3.8 and 3.3 mg. per hundred cubic centimeters.

In December 1940 the patient fell down a flight of steps and fractured his skull. He died in ten hours without recovering consciousness.

CASE 3.—J. B., when first seen, in 1937, at the age of 58, stated that he was very well until about the age of 46 years when, during a game of tennis, he noticed a weakness of the right hand. The weakness of the right hand gradually progressed, and atrophy of the muscles of the forearms became evident. At about 52 years of age he noticed atrophy in his left forearm. Later his legs· became increasingly stiff and weak. The stiffness was worse after he had been sitting for some time and in cold weather. It diminished after walking. He stubbed his toes when he walked. His articulation had been poor for three to four years.

For several years the patient had had marked generalized weakness. His hands became cold easily. He had lost about 5 pounds (2.5 Kg.) in the last year, and when seen weighed about 100 pounds (45.4 Kg.). His best weight was 134 pounds (60.8 Kg.) at the age of 20 years.

He had been wearing glasses for more than fifteen years. During the last five years, his eyes had been watering.

He had measles and whooping cough in childhood, a febrile disease ("typhoid-malaria") at 19 years and influenza at 41 and 44 years of age. He was born in Texas, one of twins. The twin brother died at 8 months of age. An older brother had the same disease, as did his son and daughter.

When examined in September 1937 the patient appeared somewhat older than his stated age of 58 years. He was of medium height and of rather slight build, cooperative and intelligent. He talked in a nasal, monotonous, low-pitched voice, at times difficult to understand. He walked with a definite "steppage" gait, with

the body flexed forward. The skin over the face was tight, thin and shiny. He was bald, and his teeth had been extracted. On examination the thyroid gland was not enlarged; the lungs and the abdomen were normal, and the testicles were definitely smaller and softer than normal.

The eyes showed chronic conjunctivitis. The cornea, the anterior chamber and the iris of each eye were normal. The lens of each eye showed early subcapsular and posterior starlike opacities. Numerous small punctate opacities of various sizes occurred throughout the entire lens but were more numerous under the anterior and posterior capsules. With a slightly minus lens, the vision was normal.

The heart and the aorta were of normal size and shape as observed in the roentgenogram. At the apex the first sound was of moderate intensity and was followed by a rather rough, fairly loud, high-pitched systolic murmur. The blood pressure was 104 systolic and 70 diastolic. The electrocardiogram was normal.

As to the neuromuscular system, the temporal muscles were markedly atrophic; the masseter muscles, less so. The periorbital muscles, the muscles of the cheeks and the orbicularis oris muscle were weak and atrophic, producing the typical myopathic facies. Only a few fibers of the sternocleidomastoid muscles were left. All the muscles of the shoulder girdle and of the trunk showed moderate atrophy. The triceps and biceps muscles and the muscles of the forearms and hands on each side were markedly atrophied. Flexion and extension of the thigh were fairly good. Flexion and extension of the legs were weak. Dorsiflexion of the feet was very weak. All the muscles of the lower extremities were somewhat atrophic. Voluntary myotonia was present in the abductor muscles of the thumbs. All the muscles showed a somewhat increased mechanical irritability. Mechanical myotonia was present in the chin muscles, the tongue, the deltoid muscles, the extensor muscles of the fingers and wrists, the thenar and hypothenar muscles, the gluteal muscles and the calf muscles. Electrical testing revealed a myotonic reaction, modified by the atrophy. The patellar reflexes were present. The achilles tendon reflex could not be obtained. Chvostek's and Trousseau's signs were lacking.

The Wassermann and the Eagle test of the blood were negative. The urine and the blood were normal. The basal metabolic rate in June 1937 was —19 per cent. On June 19 the calcium and the phosphorus (inorganic) content of the blood were 9.8 and 4.8 mg. per hundred cubic centimeters, respectively; on June 30 they were 9.1 and 6.3 mg., and on July 1 they were 9.4 and 4.7 mg. On June 19 the chloride content of the plasma (as sodium chloride) was 632 mg., and the cholesterol content of the blood was 167 mg., per hundred cubic centimeters.

From the time he was first examined until 1942, the patient was seen at frequent intervals, and many therapeutic agents were tried. He slowly lost weight and strength and had increasing difficulty in swallowing. Aspiration pneumonia finally resulted in his death in October 1942.

Cases 2 and 3 serve to emphasize the almost monotonous uniformity of the case histories of well developed dystrophia myotonica.

CASE 4 (previously reported by Moleen, Johnson and Dixon [1]).—L. B. L. was first seen at the age of 35 and followed to his death at the age of 56. Two of his brothers were similarly affected. One of these died at the age of 60; no autopsy was done, and the cause of death was stated in the hospital record as myocarditis and auricular fibrillation. The case of the other affected brother is presented as case 5. Another brother was well in 1939, at the age of 63. A somewhat younger brother was well when last heard of, several years ago. One brother died at 19 years of age, of "lead poisoning." A sister died at 64 years of age, of "inward

goiter." Their father died at about 55 years, of "pneumonia," and their mother died at 74 years, of "cancer of the uterus" and "old age."

When seen at the age of 35, the patient stated that at about the age of 27 he noticed a loss of power in the hands and arms, which became progressively weaker and thinner. After about four years, weakness of the legs became evident and likewise progressed steadily. No numbness, tingling or pain was noticed at any time. An occasional twitching of the muscles was noticed. There was no subjective difficulty of vision. Examination at that time revealed an anemic, slender white man with no evident defect of speech. The volume of the tongue was reduced. Symmetric wasting was present throughout the body, but was more evident in the hands, arms and shoulders. The thenar and hypothenar eminences and all the intrinsic mucles of the hands were distinctly wasted. Muscular power in the grasp was greatly reduced. The flexor and extensor power in the arms was markedly diminished. No spontaneous fibrillation was noted, but tapping the pectoral muscle caused a rhythmic wavelike contraction from insertion to origin. In walking, the patient raised the feet higher than was normal in order to clear the toe; the foot was carried forward and struck the floor flat. No ataxia was evident in the movements of the arm or the trunk. Tendon reflexes were generally diminished, but present, and no abnormal reflexes were seen. All forms of sense perception were normal. Visual acuity was normal.

When the patient was examined four years later, at the age of 39, a progression of the condition was observed. A slight difficulty in articulate speech was recognized, especially in cold weather, and the patient stated that the muscles of the tongue would not move as well as before. On an attempt to drink while in a horizontal position, the water ran out of the nose, and if he drank hurriedly, he invariably strangled. The movements of the head were notably weakened, and it had often been necessary for him to assist with his hand when raising his head from the pillow. The depressor muscles of the jaw were weak, and the temporal, pterygoid and masseter muscles were markedly weakened. Wasting of the temporal muscles was definite. The grasp was weaker than when the patient was first seen. The intrinsic muscles of the hands showed more wasting than before, and the eminences were less prominent. The patient found difficulty in relaxing the grasp, the extensor muscles "being too weak to overcome the contraction of the flexors." Flexion of the leg was performed with greater power than extension. Dorsiflexion of the feet was accomplished only with difficulty, and when dependent the feet hung in a position of plantar flexion, or foot drop. Tapping the quadriceps or the sartorius muscle caused a "myotatic" response. The reflexes were more feeble than on the first examination. Tapping the right forearm extensor tendon caused a slight extension of the fingers, which slowly relaxed. Vision had decreased to 15/20 on the right and 15/30 on the left. All forms of sensory perception were normal.

The course of the disease was one of gradual progression, and death occurred at the age of 56 of what was believed to be complete exhaustion.

CASE 5.—In the distribution of the weakness and atrophy and the course of the disease this patient, G. L., was very similar to his brother (case 4). He dated the onset of his weakness from an injury sustained by him at 21 or 22 years of age, when he fell backward over a snow plow and struck his back. The weakness was first noticed in his hands and wrists.

At the age of 40 the patient presented moderate atrophy. The strength of the extensor muscles was definitely weaker than that of the flexor muscles. The tongue was somewhat shriveled in appearance. The reflexes were diminished in degree. Special senses were unimpaired.

The weakness and atrophy of the muscles progressed, and when he was admitted to the hospital at the age of 49 years for bronchopneumonia, the atrophy of the muscles of both upper and lower extremities was fairly marked. At the age of 56 years he was markedly emaciated, and most of the muscles of the body, especially those of the forearms, legs and back, were markedly atrophied. At no time was there any pain or sensory disturbance. He died at the age of 59 of bronchopneumonia.

The conclusion that the last 2 patients had dystrophia myotonica is based on the following evidence: 1. The type of heredity is that found in patients with dystrophia myotonica and differs from that found in patients with other muscular atrophies that occur in adults. Three of the six siblings who lived long enough to manifest the disease were affected. This 50 per cent incidence is commonly seen in families with dystrophia myotonica and is one reason why we believe the disease to be transmitted as a dominant character. The cause of the usual failure to recognize the disease in the parents has been referred to. 2. The distribution of the atrophy was characteristic of dystrophia myotonica. In both patients the atrophy was first noticed in the forearms and hands. Foot drop and inability to bring the head up when lying down were also noted early. The muscles to which these signs are referable will be recognized as the ones which characteristically show early involvement in dystrophia myotonica. The generalized atrophy which occurs as the disease progresses is also characteristic. 3. Voluntary myotonia was present. It is stated that patient L. B. L. (case 4) found difficulty in relaxing his grasp of objects. This difficulty cannot occur if the flexor muscles relax normally, even if the extensor muscles are weak. It occurs only when the contraction of the flexor muscles persists for an abnormally long time after cessation of voluntary effort at contraction—that is, when voluntary myotonia is present. 4. Mechanical myotonia was present. On one examination it was stated that tapping the forearm extensor tendon caused a slight extension of the fingers which slowly relaxed. This is a clear description of a form of mechanical myotonia which can be duplicated in almost any patient with dystrophia myotonica. 5. Some of the dystrophic and endocrine changes which ordinarily complete the picture of dystrophia myotonica were present. These include baldness in both patients and testicular atrophy in L. B. L. (case 4).

AUTOPSY REPORTS AND HISTOLOGIC STUDIES

CASE 1 (F. B., aged 44).—This is an abstract of the autopsy record (significant observations only are mentioned).

Gross Findings.—The body was that of a white man; the weight was 168 pounds (76 Kg.) and the length 5 feet 11 inches (180 cm.). The state of nutrition was observed to be fairly good. The atrophy of the sternocleidomastoid muscles and of the muscles of the forearms and hands was found to be well marked. Rigor and livor mortis were slight nine and one-half hours after death. The right pleural cavity contained 200 cc. of clear greenish fluid; the left was empty. Both were free from adhesions. The thyroid gland was small and flattened. The four

parathyroid glands were normally situated but were small, each being 0.3 cm. in the greatest diameter. Several small cysts (0.2 to 0.5 cm. in diameter) were located in the loose connective tissue surrounding the parathyroid glands. The heart weighed 420 Gm. and exhibited no gross abnormalities. Both lungs were large and heavy. The bronchi contained mucopurulent secretion. The surfaces of cut sections of both lungs were very moist and showed purplish red patches on a pink background. The lower lobe of the right lung contained two peripherally located pyramidal grayish solid zones, each about 2 cm. in diameter. The spleen weighed 450 Gm. and was about twice normal size. The splenic substance was dark red, soft and bulging, with numerous white dots scattered throughout. The gastrointestinal tract was normal except for scattered petechial hemorrhages in the pyloric and duodenal mucosa. Both adrenal glands were small, with thin cortical layers. The right weighed 4 Gm. and the left 5 Gm. The testicles were small and soft. Each measured 4.5 by 1.3 by 1.3 cm. Cut surfaces were not grossly remarkable. The skeletal muscles varied from those that were nearly normal in appearance, such as the deltoid, pectoral, abdominal and thigh muscles, to those that were partially or almost completely atrophic. In the latter group were the temporal, the sternocleidomastoid and the arm, forearm and leg extensor groups. These were of a peculiar grayish white color streaked with yellow and were more or less reduced in volume. The temporal and sternocleidomastoid muscles were scarcely recognizable as muscles, being reduced to flat fibrous strands. The peripheral nerves, the brain and the spinal cord were grossly normal. The brain weighed 1,365 Gm. The pituitary gland appeared normal and weighed 0.45 Gm.

Histologic Examination.—The tissues were fixed in Zenker's solution and embedded in paraffin; sections were stained with hematoxylin and eosin unless otherwise stated. Thyroid Gland: The acini were less numerous and more variable in size than those of a normal gland and were lined with low cuboidal epithelium. All were filled with blush-staining colloid. The stroma was fibrous and appeared slightly increased in amount. The appearance was similar to that of senile atrophy.

Parathyroid Glands: In all the glands the stroma showed partial adipose tissue replacement. The parenchyma was composed of "chief" cells, which in some areas formed small acini filled with dull bluish hyaline material. Oxyphilic cells were fairly numerous but lacked regular arrangement. Small cysts lying in the surrounding adipose tissue had a thin fibrous wall without distinct epithelium and a hyaline eosinophilic content.

Heart: Sections of right and left ventricular myocardium showed moderate variability in size of fibers with variation in nuclear size and shape. Cross striations were well defined in all fibers. The number of nuclei was not increased over the normal, and clumping of nuclei as seen in skeletal muscle was lacking.

Lungs: The bronchi were filled with mucus and leukocytes. There was patchy consolidation with acute inflammatory exudate in the alveoli. The peripheral zones of discoloration seen grossly in the lower lobe of the right lung were infarcts, one of which was undergoing marginal organization. There was an unorganized thrombus in the right main pulmonary artery.

Spleen: The sinusoids contained erythrocytes, while the pulp spaces were crowded with erythrocytes and leukocytes of various types, with many polymorphonuclear neutrophils and eosinophils. The malpighian corpuscles were numerous and large.

Gastrointestinal Tract: In sections of the upper end of the esophagus the muscle fibers showed abnormalities characteristic of the disease (fig. 1 *A*). Cross sections of the fibers were circular or oval and varied from 20 to 140 microns in diameter.

The nuclei were apparently increased in number and irregular in shape, and many lay in the substance of the fiber, occasionally in clumps. Many of the larger fibers had a light brownish staining reaction. In these the cut ends of the myofibrils were separated by hyaline substance, and myofibril grouping into Cohnheim's fields was not visible. Cross striations were fully evident except in the large hyalinized fibers, where they sometimes were and sometimes were not visible. When present in these fibers the cross striations were less distinct, more delicate and more closely spaced than those in the other fibers. The sarcolemma might either closely invest the fiber or be completely separated from it. The perimysium was relatively increased in prominence. There was no evidence of inflammation or fibrosis.

Skeletal Muscles: The individual muscle specimens were placed in separate containers at autopsy in order to insure their correct identification. All varied in
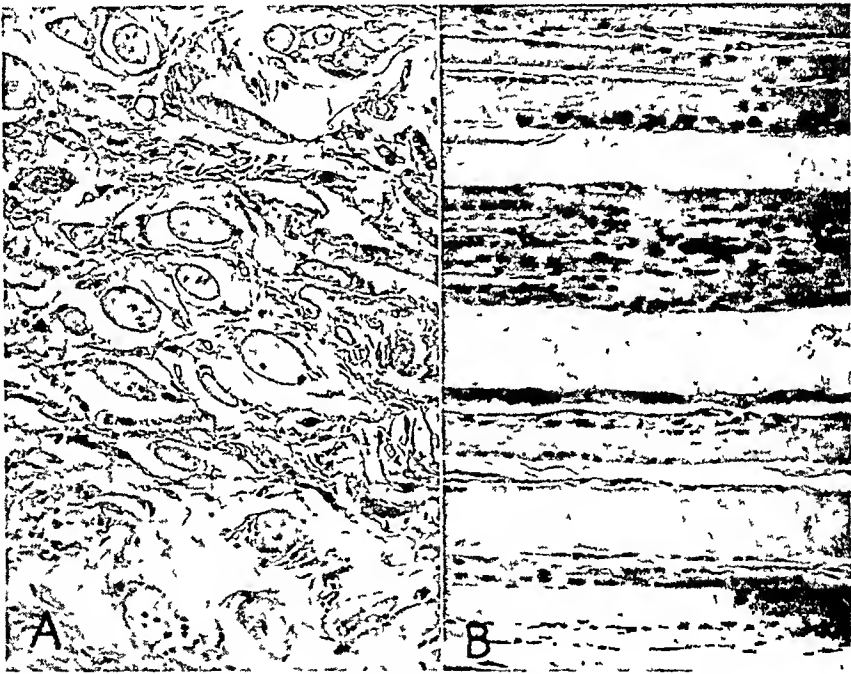
Fig. 1 (case 1).—*A*, cross section of muscle of the upper part of the esophagus; × 125. *B*, flexor muscle of the forearm; × 125.

some degree from the normal, with the exception of the diaphragm, in which no definite changes of muscle fibers were seen. There was, however, a chronic inflammatory reaction along the pleural border. Increasingly severe involvement was observed in the following muscles or muscle groups in the order named: deltoid; quadriceps femoris; pectoralis major; rectus abdominis; arm flexor; leg flexor, extensor and peroneal; arm extensor; temporals and sternocleidomastoid.

In the first four muscles much of the tissue appeared histologically normal, with only an occasional fiber or small group of them being visibly altered. In these the most striking feature was an increase in the number and an alteration in the position of the nuclei. The involved fiber might be increased, normal or diminished in size The nuclei lay in the sarcoplasm away from the sarcolemma and frequently were arranged in rows along the long axis of the fiber. Cross striations were visible and might be coarse but were often delicate and closely

spaced (fig. 1 *B*). The lesion might be noted at separate points along a single fiber, with normal-appearing intervening structure. Occasionally a coarsely vacuolated fiber was seen.

In the other muscles studied, the amount of tissue involved was greater, and the lesions were more severe. As the process became more marked, the fibers became rounded as seen in cross section. While some were normal or increased in diameter, the majority were smaller, ranging from 20 to 120 microns in diameter. The nuclei showed variation in intensity of staining, and in the advanced lesions many were hyperchromatic. Cohnheim's fields disappeared in many fibers about the periphery, persisting in the central zone. In other instances the cross section appeared hyaline or vacuolated, no myofibrils being visible. The substance of such fibers stained a deeper, faintly bluish pink. An extreme degree of involvement was seen in the temporal muscle. Here the fibers were small, although giant fibers were present, with long rows of hyperchromatic nuclei, which were often clumped. Most of these retained both cross striation and visible myofibrils. Segments of involved fibers might be thinned out to filament dimensions yet retain cross striation throughout. Fatty metamorphosis was not noted in any of the skeletal muscles. The blood vessels appeared normal except for certain small veins in the muscles of the lower extremities in which recent thrombosis had taken place. By the use of Bielschowsky's and of Bodian's staining methods, the small myelinated nerve trunks within the muscles were seen to be somewhat lacking in number of both myelinated and nonmyelinated fibers as compared with the normal. Nonmyelinated nerve fibrils ramified throughout both the affected and the nonaffected muscles. No relationship between distribution of nerve fibrils and normal or atrophic muscle fibers could be established.

Spleen: The organ appeared congested. The red pulp contained numerous leukocytes, many of which were eosinophilic polymorphonuclears.

Liver: No significant changes were present.

Pancreas: This organ appeared normal.

Adrenal Glands: Both glands were alike. The cortices were reduced to approximately half the normal thickness. There was reduction in number of cells in the fascicular zone, with lack of lipid storage. Irregularly spaced groups of fascicular cells had coarsely vacuolated cytoplasm. Those nearest the medullary border contained brown granular pigment. Single cells and groups of them appeared hypertrophied; others (the remainder), more or less atrophic. The glomerular layer was uneven; it appeared atrophic, with zones of cellular hypertrophy. The cells were hyperchromatic and more deeply basophilic than the normal (compared with those of a man of the same age killed in an automobile accident). The zona reticularis was poorly defined and variable in thickness. In places it underlay the glomerular zone directly, owing to the apparent disappearance of the fascicular layer. Cells contained brownish granular pigment, and occasional cells were vacuolated. "Dark" cells predominated. Pyknosis of nuclei with shrinkage of cells was evident along the medullary border (fig. 2 *A*).

Kidneys: These were essentially normal.

Urinary Bladder: This was normal.

Prostate: This was not remarkable.

Testicles: The right testicle contained a few atrophic tubules, but for the most part the tubules were normal and contained spermatozoa. The left showed interstitial edema, with only a few atrophic tubules among the normal ones. There was an apparent increase in number of interstitial cells (fig. 2 *B*). The right epididymis was normal. The left contained calcified debris within the ducts.

Pituitary Gland: The fibers of the posterior lobe were less compact than usual, with granular material present in the interstices. The pars intermedia contained

several "colloid"-filled cysts. There was an apparent reduction of the total number of cells in the anterior lobe. The cells formed acini, which in many instances were filled with solid plugs of basophilic hyaline substance. Basophilic cells predominated with only an occasional eosinophilic cell present (fig. 3 A).

Cervical Ganglions: These appeared normal.

Bone (vertebra and rib): These specimens appeared normal.

Pineal Gland: This was anatomically normal, with the usual number of calcospherites lodged within it.

Central Nervous System: There were no significant alterations in the brain. The ganglion cells of the anterior horn appeared to be reduced in number.

Nerve Trunks: The medium-sized trunks (posterior tibial, for instance) appeared to be somewhat deficient in number of nerve fibers.

Sympathetic Ganglions: These appeared normal.

Fig. 2 (case 1).—A, adrenal cortex; $\times$ 125. B, testis; $\times$ 125.

CASE 2 (O. M., aged 44).—This autopsy was performed at the Denver General Hospital. The body is stated to have been well developed and well nourished. There is no statement regarding muscle wasting and no description of the external genitalia. The heart weighed 340 Gm. and appeared normal grossly. The adrenal glands were normal except for a cortical adenoma, 1 cm. in diameter, present in the left gland.

The brain weighed 1,350 Gm. There was extensive subdural hemorrhage, accompanying skull fracture. The pituitary gland, the thyroid gland and the spinal cord are not described grossly. Microscopically, the brain showed no changes except those produced by recent trauma. The pituitary gland appeared normal. The adrenal gland was normal except for the presence of the cortical adenoma.

There were two sections of skeletal muscle, one of which appeared entirely normal. The other showed fibers varying in size from a few larger than normal in diameter to many which were smaller than normal. Tapering, wavy and segmentally constricted fibers were seen. There was an increase in the number of

nuclei, and some were arranged in rows apparently within the sarcoplasm. Cross striations were present except in a few of the wavy fibers in which the sarcoplasm was hyalinized. Clumping of nuclei was not observed. The muscle changes were of the same type as in the other cases.

CASE 3 (J. B., aged 63).—The body was emaciated, with very marked wasting of the muscles of the face, the neck, the shoulder girdle and the extremities. Atrophy of the tongue, the extrinsic muscles of the larynx and the skeletal muscle of the upper portion of the esophagus was present. The heart was essentially normal. There was a moderate degree of atherosclerosis of the coronary arteries and the aorta. Both lungs were heavy and wet; the cut surfaces were a mottled purplish red and exuded much watery pink fluid. The bronchi were filled with thick mucopurulent secretion. The adrenal glands together, with some attached adipose tissue, weighed 9 Gm. The cut surfaces showed thin, uneven, poorly
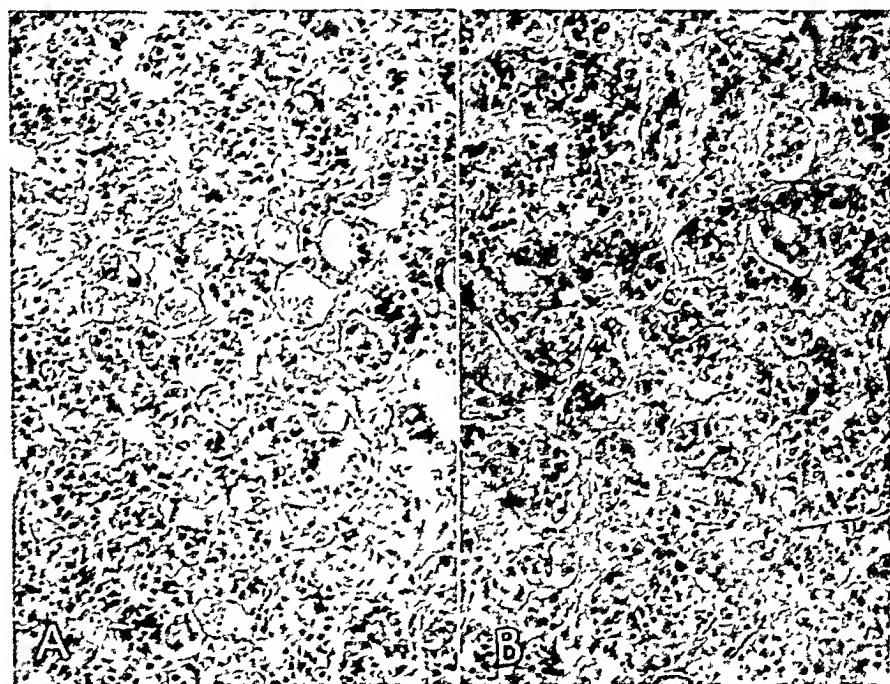
Fig. 3.—*A*, anterior lobe of the hypophysis in case 1; × 125. *B*, anterior lobe of the hypophysis in case 3; × 125.

demarcated yellow cortices. The prostate was small and soft. Both testicles were small and very soft; each weighed 30 Gm. The brain and the spinal cord were grossly normal. The pituitary gland weighed 0.5 Gm.

Microscopically, changes in the skeletal muscles were identical with those found in the other cases. The thyroid and parathyroid glands were partially atrophic. The thymus showed complete involution. The lungs showed confluent bronchopneumonia with foreign material (cellulose fibers) in the bronchi and alveoli, indicating that aspiration of food particles had occurred. The adrenal glands showed cortical atrophy with patchy hyperplasia of the zona glomerulosa and the zona fascicularis and irregular lipid storage. The testes were completely atrophic and did not show the apparent increase in interstitial cells observed in the other cases. The prostate was entirely lacking in glandular hyperplasia. The anterior lobe of the pituitary gland was sharply congested, with fewer than the usual number of cells, but all cell types were present and appeared in approximately

normal distribution (fig. 3 *B*). Hyalin-filled or "colloid"-filled acini were numerous. The posterior lobe appeared normal.

CASE 4 (L. B. L., aged 56; case reported by Moleen, Johnson and Dixon [1]).— The body showed extreme emaciation with marked muscle wasting, but the heart weighed 310 Gm. and was normal in size and shape. The right adrenal gland weighed 8 Gm.; the left 8.5 Gm. The cortices were paler and thinner than they are normally. The testicles were small and soft. The lumbar enlargement of the spinal cord was soft and shrunken. The pituitary gland was small; the weight was not recorded. A gross description of the thyroid gland is not given; microscopically the gland was found to contain several large colloid-filled cysts. The remainder of the gland was made up of large acini solidly filled with colloid, lined by low cuboidal epithelium and apparently inactive.

The changes in the skeletal muscles differed in no way from those described in case 1. The cardiac muscle did not exhibit any such changes. The adrenal glands both showed decreased cortical cellularity. The number of cells in the zona glomerulosa and the zona fasciculata was reduced, with lipid-bearing cells arranged in groups. The zona reticularis was broad, but not compact, with many cells containing light brown pigment. The medulla of each appeared normal. The spinal cord and peripheral nerve changes have been described in detail by Dixon,[1] who expressed the belief that they were the primary pathologic lesion. ("Non-inflammatory degeneration of the myelinated elements of the peripheral nerves appears to constitute the primary pathologic lesion.") Guillain, Bertrand and Rouques [8] expressed the belief that the nerve changes are secondary.

The pituitary gland was not entirely normal. There was partial fibrosis, with reduction in number of cells of the anterior lobe and relatively greater reduction in number of the acidophillic cells.

The testicles were practically completely atrophic.

CASE 5 (G. L., aged 59).—There was marked emaciation with great wasting of muscles. The testicles were about one-half normal size. The heart weighed 250 Gm. and was essentially normal in gross appearance. Both lungs were heavy, with patchy consolidation throughout. The adrenal glands were normal grossly (weight not recorded). The thyroid gland, the pituitary gland, the brain, the spinal cord and the testicles are not described.

The skeletal muscle changes were similar to those observed in the other cases. The heart muscle showed an increased amount of lipochrome pigment but was otherwise normal. The lungs showed confluent bronchopneumonia. In the adrenal glands there was a reduction in thickness of the zona fasciculata, with well defined island grouping of cells containing lipoid material, but the degree of alteration was not so great as in some of the other cases. The testicles showed almost complete atrophy, only a few seminiferous tubules remaining, with no evidence of spermatogenesis.

Sections of several nerve trunks all showed reduction in number of myelinated fibers.

There were no sections of brain, spinal cord or pituitary gland.

COMMENT AND SUMMARY

Five cases of dystrophia myotonica were studied pathologically so far as tissues were available. In one instance the central nervous system was not examined, and in another not all of the organs were

8. Guillain, G.; Bertrand, I., and Rouques, L.: Ann. de méd. **31**:180, 1932.

saved for microscopic study. Consequently the cases cannot be pre-presented on a fully comparative basis. However, we believe that enough data have been obtained to justify a report.

In addition to the lesion of the skeletal muscles a number of investigators have described changes occurring in the endocrine glands and the central and peripheral nervous systems. These changes occur but are inconstant and do not permit interpretation at the present time.

In 4 of the 5 cases the pituitary gland was available for study. In 2 instances the anterior lobe was somewhat atrophic, with a relative increase in number of basophilic cells. In the other 2 the gland was apparently within normal limits.

In 4 of the 5 cases the adrenal glands showed a degree of cortical atrophy, particularly affecting the zone fasciculata, with island grouping of cells containing lipoid material. In the fifth case the adrenal glands were normal except for a cortical adenoma of the left gland.

In 4 cases the thyroid gland appeared to be less than normally active. In the remaining case it was not available for study.

The brain and the spinal cord were studied in 3 cases (1, 3 and 4). The spinal cords appeared deficient in number of anterior horn cells, with segmental differences in number of these cells. Peripheral nerve trunks were studied in 3 cases, and were found to be deficient in number of myelinated nerve fibers.

An effort was made in 1 case to demonstrate the nonmyelinated nerve fibrils and motor end plates of skeletal muscles. The results were not entirely satisfactory because of technical difficulties. However, nonmyelinated fibrils were observed to ramify equally in affected and nonaffected muscles, and motor end plates as well as sensory endings were found in muscles in all stages of involvement.

The testicles were almost completely atrophic in 3 cases, slightly atrophic in 1 and were not studied in 1 case.

The muscle lesion is characterized by irregularity of distribution within the perimysium, by apparent increase in number of nuclei, by alteration in spacing and location of nuclei and by striking changes in size of individual fibers. The late changes include fibrosis and adipose tissue replacement and are considered to be secondary.

No explanation of the disease can be advanced from a pathologic standpoint, although it may be stated that there is associated hypoplasia or partial atrophy of the anterior lobe of the pituitary gland, the thyroid gland, the adrenal cortex and the testicles.

The fact that severe bronchopneumonia was present in 3 of the patients who died naturally suggests that bronchopneumonia is a common terminal event, to which the patients predispose themselves when they aspirate food and other material from the pharynx as a result of myotonic changes in the muscles of deglutition.

Case Reports

MIXED SQUAMOUS CELL CARCINOMA AND PAPILLARY ADENOCARCINOMA (ADENOACANTHOMA) OF THE THYROID GLAND

RODERICK C. ROSS, M.D.
MONTREAL, CANADA

CANCER of the thyroid gland is infrequent, occurring in 1 to 2 per cent of thyroid glands removed surgically. In a series of 3,389 thyroidectomies at the Lahey Clinic, 1916 to 1937, Clute and Smith[1] found 67 cancers, an incidence of 1.68 per cent. Squamous cell carcinoma of the thyroid gland is rare. In 774 cases of cancer of the thyroid gland reported from the Mayo Clinic in the period from 1907 to 1937 by Pemberton[2] the frequency was: papillary adenocarcinoma, 30 per cent; adenocarcinoma in adenoma (cancerous adenoma), 38.1 per cent; diffuse adenocarcinoma, 30.4 per cent; sarcoma, 0.75 per cent, and squamous cell carcinoma, 0.75 per cent. Davis[3] reported 2 cases of squamous cell epithelioma in a series of 50 cases of cancer of the thyroid gland. Other reports of cases of carcinoma of the thyroid gland include single cases of squamous cell carcinoma.[4]

The simultaneous occurrence of squamous cell carcinoma and any other type of carcinoma of the thyroid gland has not been reported. A case of mixed squamous cell carcinoma and papillary adenocarcinoma is therefore considered of sufficient interest to report.

REPORT OF A CASE

This 61 year old Greek man was admitted to the Royal Victoria Hospital, Montreal, April 7, 1946, complaining of anginal attacks and dyspnea of three years' duration, hoarseness of three months' and hemoptysis of two months' duration. He had been hospitalized on two previous occasions for "heart trouble" and gallstones.

In January 1946 his throat became sore and hoarseness developed. Shortly after, hemoptysis appeared, and paralysis of the right vocal cord was discovered. In March fixation of the larynx was noted, and the impression was that of a neoplasm of the right lobe of the thyroid gland.

On his admission to the hospital, examination revealed a blood pressure of 138 systolic and 70 diastolic, a palpable liver and spleen and paralysis of the right vocal cord. Bronchoscopy showed a normal trachea and bronchi. A firm nodule was palpable in the right lobe of the thyroid gland, but surgical removal was considered inadvisable. The basal metabolic rate was +1 per cent. He was discharged on April 25 as improved.

---

1. Clute, H. M., and Smith, L. W.: Arch. Surg. **18**:1, 1929.
2. Pemberton, J. de J.. Surg., Gynec. & Obst. **69**:417, 1939.
3. Davis, H. A.: Arch. Surg. **39**:435, 1939.
4. Smith, L. W.; Pool, E. H., and Olcott, C. T.: Am. J. Cancer **20**:1, 1934. Clute and Smith.[1]

His final admission was on September 12. At this time he complained of expiratory dyspnea of two weeks' duration, which had increased markedly on September 7.

His breathing was labored, particularly on inspiration, and stridor was present. On the right side of the neck, just above the medial extremity of the clavicle, a firm mass, ¾ inch (about 2 cm.) in diameter, was palpable. The diagnostic impression was that of mechanical obstruction of the larynx or the trachea. A hemogram on September 14 showed red blood cells, 5,400,000; hemoglobin, 106 per cent; white blood cells, 7,400, and sedimentation rate, 27 mm. in one hour (29 corrected as to cell volume.)

On September 17 bronchoscopy was performed. One-half inch below the vocal cords there was a mass projecting into the trachea from the right side, nodular and vascular in appearance, filling the trachea except for a slit 2 mm. in width along the left wall. As soon as the mass was touched, it began to ooze. A biopsy was made, after which the patient stopped breathing and had to be intubated. Tracheotomy was then carried out. The impression was that of carcinoma involving the right lobe of the thyroid gland and secondary infiltration of the trachea.

The same evening the patient complained of a choking sensation; suction was used, but the tracheotomy tube did not appear to be blocked. His face became swollen and cyanotic, and within fifteen minutes he died.

Autopsy (fourteen and one-half hours post mortem; limited to thorax and abdomen).—Only those findings pertinent to the case will be described in detail.

The face was livid and swollen. Interstitial emphysema was present in the neck and the mediastinum. A tracheotomy tube was lying in the interstitial tissue anterior to the trachea. A small amount of blood was present in the trachea.

The right lobe of the thyroid gland was one and a half times the size of the left lobe, firm in consistency and adherent to the surrounding structures. On section most of the peripheral portion was seen to consist of normal tissue, but medially the gland had been replaced by an irregular nodular mass, 3 cm. in diameter. It was firm in consistency and white with small yellow areas. Posteriorly the nodule extended to the capsule of the gland but had not penetrated it. Medially the growth had ulcerated into the trachea just inferior to the cricoid cartilage, and within the trachea there was a reddish, friable mass, 1.5 by 1 by 1 cm. A tracheotomy incision was present, immediately inferior to the mass in the trachea. The right recurrent laryngeal nerve was traced upward and lost in the tumor mass. The left lobe of the thyroid gland was reddish brown and moderately firm in consistency and on section showed normal structure.

The heart showed hypertrophy of the left ventricle (460 Gm.) and fibrosis of the interventricular septum. Moderate coronary arteriosclerosis was present, involving especially the anterior descending branch, but no occlusion was demonstrated. There was chronic passive congestion of the lungs, the liver, the spleen and the kidneys. No metastases of tumor were found.

Incidental findings included cholelithiasis, obsolete tuberculosis of bronchial lymph nodes and of the spleen, and hyperplasia of the prostate.

Microscopic Observations.—The right lobe of the thyroid gland contained a small amount of relatively normal tissue immediately beneath the capsule. The acini varied considerably in size and were well filled with eosinophilic colloid. There was a slight increase of interstitial fibrous tissue. In a region more remote from the capsule, the acini were poorly filled with pale colloid, and there was a moderate increase in hyaline connective tissue with calcification, small areas of hemorrhage and focal collections of lymphocytes and plasma cells.
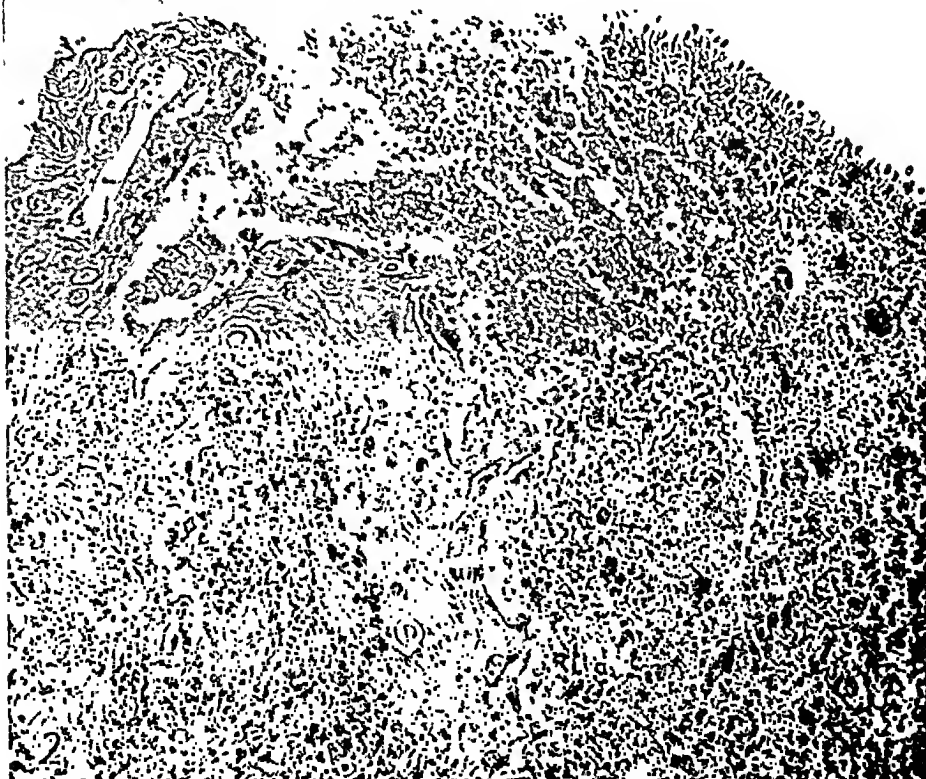
Fig. 1.—Photomicrograph of tumor in the right lobe of the thyroid gland showing squamous cell carcinoma with pearl formation in the lower half of the field and papillary adenocarcinoma in the upper half. Central necrosis is evident in the squamous cell carcinoma. Hematoxylin-eosin. × 110.

Fig. 2.—Photomicrograph of tumor in the area of tracheal ulceration showing tracheal epithelium, in the upper right of the field, which is interrupted by squamous cell carcinoma, with no evidence of transition. Hematoxylin-eosin. × 112.

194

Blending with this altered thyroid tissue were two distinct types of neoplastic epithelium and intermediate gradations. The predominant type consisted of large, irregular acini with papillary projections, lined by darkly staining high columnar epithelium. The nuclei, which were located near the basement membrane, were elongated, hyperchromatic and vesicular, and showed few mitotic figures. The second type, comprising 40 to 50 per cent of the tumor, consisted of islands of keratinizing squamous epithelium with pearl formation and areas of central necrosis. The nuclei were large and vesicular, with distinct nucleoli. The cytoplasm was pale staining and vacuolated, and intercellular bridges were present. In other areas there were large pleomorphic cells with one or more very large vesicular or hyperchromatic nuclei and faintly basophilic cytoplasm, which trailed off into processes and in some cases contained phagocytosed polymorphonuclear leukocytes. In areas of the tumor lying between adenocarcinoma and squamous cell carcinoma acini were present which showed piling up of epithelium to a varying degree, but it was not possible to demonstrate an acinus that was clearly lined by columnar epithelium in one area and by stratified squamous epithelium in another (fig. 1).

The stroma varied in different portions of the tumor. In the acinous areas it occurred as fibrous septums between groups of acini, and in the squamous areas, as dense collagenous connective tissue surrounding larger or smaller islands of epithelial cells. There was a moderate infiltration of lymphocytes, plasma cells and polymorphonuclear leukocytes, the latter in relation to areas of necrosis.

Section of the area of tracheal ulceration showed the pseudostratified ciliated columnar epithelium of the trachea interrupted by keratinizing squamous cell carcinoma penetrating from without (fig. 2). All the tumor tissue which projected into the trachea was of the squamous cell type, but papillary adenocarcinoma was in close relationship and lay between the cartilage of the tracheal ring and the mucosa. At one point the mucosa overlying the adenocarcinoma was necrotic. There were hyperemia, small areas of hemorrhage and a marked infiltration of polymorphonuclear leukocytes, plasma cells and eosinophilic leukocytes in the submucosa of the trachea and the projecting portion of tumor.

Although the tumor lay in close relationship to capillaries, no invasion of veins or lymphatic channels was observed and sections of the regional lymph nodes contained no tumor.

In the left lobe of the thyroid gland the acini were fairly uniform in size, lined by low cuboidal epithelium and well filled with lamellated eosinophilic colloid. The capsule was normal, and the interstitial tissue and vessels showed no abnormality.

COMMENT

This case was interpreted as one of mixed squamous cell carcinoma and papillary adenocarcinoma of the thyroid gland with local invasion and ulceration of the trachea. The possibility that the squamous cell carcinoma may have originated from respiratory epithelium which had undergone squamous cell metaplasia must, however, be considered.

Carcinoma of the trachea is a rare type of neoplasm, but of 95 cases that have been described [5] 42 were of the squamous cell type. However, by distribution, only 35 per cent of the lesions occurred in the upper third, and of these more than one half were on the posterior wall of the trachea. Thus the site of the tumor in this case is not a common site of tracheal carcinoma.

---

5. Olsen, A. M.: Arch. Otolaryng. **30**:615, 1939.

However, one of the most significant points against primary squamous cell carcinoma of the trachea is the mode of the neoplastic ulceration of the trachea. The normal pseudostratified ciliated columnar epithelium was suddenly interrupted and inverted into the tracheal lumen by squamous cell carcinoma. There was no suggestion of gradual transition between this epithelium and the squamous cell type (fig. 2), and although the tumor which projected into the tracheal lumen was purely squamous cell in type, well differentiated papillary adenocarcinoma had invaded the tracheal wall, lying between tracheal cartilage and mucosa and at one point producing necrosis of the overlying mucosa. In addition, the main mass of squamous cell carcinoma was in the right lobe of the thyroid gland and only a relatively small portion was within the trachea.

Moreover, five months before death the trachea was normal on bronchoscopic examination, one month after a firm nodule had been discovered in the right lobe of the thyroid gland, which was fixed to the larynx. From these facts alone, spread from thyroid gland to trachea is more plausible than the reverse.

The matter of whether the squamous cell carcinoma had its origin in the thyroid gland is unsettled. Ewing [6] stated that such a lesion arises not from branchial remnants but from the thyroglossal duct and its pyramidal process.

Jaffé [7] expressed the belief that epithelial metaplasia is the source of squamous cell carcinoma of the thyroid gland, and he reported 3 cases of sclerosis of the thyroid gland in which cuboid epithelium of the follicles changed to squamous epithelium. He also reported 1 case of metastatic abscess of the thyroid gland in which islands of squamous epithelium were found, some showing evidence of central keratinization, which were connected with follicles by a single layer of flattened epithelial cells. He considered that there was no indication that the squamous epithelial cells in the thyroid gland may have been derived from embryonic structures, such as the thyroglossal duct, the ultimobranchial body or the branchial clefts. He quoted Lubarsch,[8] who stated that for metaplasia to occur it is necessary that old cells degenerate and that young and not yet fully differentiated cells proliferate and meet with changed environmental conditions. Jaffé continued that if these requirements are fulfilled in the thyroid gland, the follicular epithelium may change its character as epithelial cells do in other organs, and that if the proliferation of metaplastic epithelium assumes the properties of autochthonous growth, there will result neoplasms which suggest a dysontogenetic origin. Three cases of squamous cell carcinoma of the thyroid gland were reported by him, in one of which follicular epithelium transformed to squamous epithelium.

Englisch and Slany [9] reported an unusual epithelial tumor of the thyroid gland, consisting of acini lined by high columnar epithelium, with buds of squamous epithelium originating at the base of the former and protruding into the glandular lumen or into connective tissue.

6. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 990.

7. Jaffé, R. H.: Arch. Path. **23**:821, 1937.

8. Lubarsch, O.: Deutsche, Ztschr. f. Chir. **227**:48, 1930; cited by Jaffé.[7]

9. Englisch, E., and Slany, A.: Zentralbl. f. Chir. **63**:2830, 1936.

They considered this to be an adenocancroid (adenoacanthoma) and not metaplasia.

In the present case, careful examination of numerous sections failed to reveal all stages of transition between columnar epithelium and stratified squamous epithelium within a single acinus. It is difficult, however, to explain the variable tumor tissues on any basis other than a combination of metaplasia and neoplasia of the epithelium of the thyroid gland.

Herxheimer, in reporting a case of carcinoma of the pylorus in which there was intermingling of squamous cell carcinoma and adeno-carcinoma, suggested the name "adenoacanthoma." [10] Tumors of this type have been reported occurring in the stomach, the uterus, the breast, the gallbladder, the pancreas, the large bowel and the lung.[11] In the present case, although the two main types of neoplastic epithelium occurred in zones, with close intermingling only in the areas of apposition, perhaps the tumor is best classified as an adenoacanthoma of the thyroid gland.

SUMMARY

A case of mixed squamous cell carcinoma and papillary adenocarcinoma of the thyroid gland, with a portion extending into the trachea, is presented. The possibility that the squamous cell carcinoma may have originated in the trachea and invaded the thyroid gland is considered, but it is concluded that both types of carcinoma arose in the thyroid gland.

Whether the squamous cell carcinoma of the thyroid gland arises from embryonic remnants or as a result of metaplasia is unsettled. The present case is interpreted as an adenoacanthoma of the thyroid gland.

10. Pasternack, J. G.: Am. J. Path. **11**:541, 1935.

11. Boyd, W.: Surgical Pathology, ed. 5, Philadelphia, W. B. Saunders Company, 1942, p. 140.

PRIMARY NEOPLASMS OF THE HEART

W. B. LEACH, M.D.
MONTREAL, CANADA

ALL TUMORS of the heart are rare, and primary tumors are even more so. Perlstein,[1] Goldstein,[2] Yater[3] and Mahaim[4] have reviewed the subject extensively, and the latter, in 1945, classified and reported on 413 cases of primary myocardial and pericardial tumors which he found during an exacting study of all the available literature. Since the publication of Mahaim's report, at least 9 more cases of primary tumor of the heart have been recorded in the literature (Straus and Merliss[5]; Batchelor and Maun[6]; Anderson and Dymtryk[7]; Pratt-Thomas[8]; Woll and Vickery[9]), and these together with the 2 cases reported here bring the total to 424.

The incidence of primary tumors as determined from autopsy material has been a matter of considerable variation. Ravid and Sachs[10] found the incidence to be 0.05 per cent during a survey of their own autopsy material. However, variations from this figure have been wide, and this was explained by Straus and Merliss[5] as being due to an insufficient number of reported cases. Mahaim[4] stated that a more authentic picture of the frequency of such tumors would be obtained if the figures were based not on the general autopsy figures but on those of patients proved to have heart disease. At the Winnipeg General Hospital during the past ten years 648 nonviable fetuses have been examined, totaling 1.4 per cent of all surgical specimens examined, and no cardiac neoplasms have been encountered apart from the one reported here. Since 1916, in the same hospital, 6,275 autopsies have been conducted, including 509 made on viable fetuses and newborn infants, and only 1 primary tumor of the heart was recorded

---

1. Perlstein, L.: Am. J. M. Sc. **156**:214, 1918.

2. Goldstein, H. I.: New York M. J. **115**:97, 1922.

3. Yater, W. M.: Arch. Int. Med. **48**:627, 1931.

4. Mahaim, I.: Les tumeurs et les polpes du coeur: Etude anatomoclinique, Paris, Masson & Cie, 1945.

5. Straus, R., and Merliss, R.: Arch. Path. **39**:74, 1945.

6. Batchelor, T. M., and Maun, M. E.: Arch. Path. **39**:67, 1945.

7. Anderson, W. A. D., and Dymtryk, E. T.: Am. J. Path. **22**:337, 1946.

8. Pratt-Thomas, H. R.: Am. J. Path. **23**:189, 1947.

9. Woll, E., and Vickery, A. L.: Arch. Path. **43**:244, 1947.

10. Ravid, J. M., and Sachs. J.: Am. Heart J. **26**:385, 1943.

(case 1). With regard to malignancy, Yater[3] reported 20 per cent of his series to be cancers; the remainder were not cancerous. Larson and Sheppard[11] in 1938 reported 29 per cent to be cancers.

Primary sarcoma of the heart was reviewed by Perlstein[1] in 1918, and of all the reported cases up until that time, he considered only 30 to have been cases of true sarcoma; he added 1 case of his own. Mahaim[4] in 1945 reviewed 87 cases, and Woll and Vickery[9] have added the eighty-eighth to the list. Since primary tumors of the heart are mesoblastic in origin, the following types of sarcoma have been described: fibrosarcoma, leiomyosarcoma, rhabdomyosarcoma, pleomorphic cell sarcoma, lymphangioendothelioma and hemangioendothelioma. The last two types are of extreme rarity. The primary tumor is found most often involving the auricles of the heart, with a greater frequency of involvement of the right side.

Of the noncancerous tumors, the myxoma is probably the most common. It is a pale, soft, pedunculated tumor, most often found in the left auricle. In the past considerable discussion has arisen with regard to this tumor. Some have considered it to be a degenerating form of an organizing thrombus in which imbibition has occurred. However, Ewing[12] and many others have held that this is not the case, finding it difficult to believe that an organizing thrombus could assume the appearance of a true myxoma. A second noncancerous tumor is the fibroma, of which 37 cases have been reported. It is a small, firm tumor arising in the subendothelial tissues; it is often attached to valves and may be polypoid in type. Occasionally the characteristics of fibroma are combined with those of myxoma in the same tumor. Then the name "fibromyxoma" has been applied. Lipoma of the heart is rare, reports of only 14 cases having been found in the literature by Mahaim.

A fourth type of noncancerous tumor is the rhabdomyoma or congenital nodular glycogenic tumor as suggested by Batchelor and Maun[6] in their excellent review of tumors of this type. Yater,[3] in his review, described three main subtypes: (*a*) solitary, located near the apex of the heart, (*b*) multiple, in which moderately sized discrete nodules are scattered throughout the myocardium, and (*c*) diffuse, consisting of numerous small nodules scattered indiscriminately throughout the myocardium. The multiple and the diffuse form are frequently associated with diffuse sclerosis of the cerebral cortex, multiple neuroglial rests in the spinal meninges and embryonic nodules of the kidney. Microscopically, the cells are of an embryonal type; they are tubular in shape and attain large size. Within the cells are large glycogen-containing vacuoles and a nucleus suspended in a weblike arrangement of cytoplasmic strands. Both in these and in the cell wall may be seen the characteristic striations of striated muscle. The majority believe that these cells are embryonal in type but may reach a high degree of maturity.

11. Larson, C. P., and Sheppard, J. A.: Arch. Path. **26**:717, 1938.

12. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 187.

REPORT OF CASES

CASE 1.—*Rhabdomyosarcoma of the Heart.*—The tumor occurred in a boy aged 14 years. He was first seen on May 6, 1936 because of cough, which was more severe on lying down, slight whitish sputum and increasing fatigue of three weeks' duration. There was no history of tuberculosis. Physical examination revealed only that a few small lymph nodes were palpable in the back of the neck. Roentgenograms of the chest disclosed a bulging mass in the superior mediastinum. The blood cell count showed 8,400 white blood cells, with eosinophils amounting to 9.5 per cent; the latter subsequently fell to 4 per cent. A tentative diagnosis of Hodgkin's sarcoma was made. Shortly after admission he contracted acute tonsillitis, which improved under appropriate treatment. On June 8 roentgen therapy was instituted and continued for about three weeks. At the end of the course of treatment there was no noticeable change in the mediastinal mass, but as the patient was feeling well he was discharged from the hospital without further treatment.
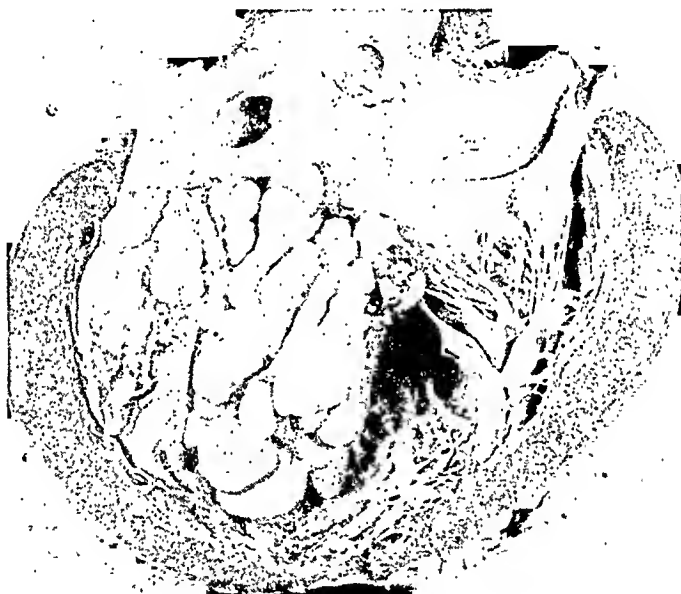
Fig. 1 (case 1).—Primary rhabdomyosarcoma of the heart involving the anterior wall of the left ventricle.

He was readmitted on August 14 with a diagnosis of bronchopneumonia. Roentgen examination of his chest showed that there had been essentially no change in the mediastinal mass since the last examination, and in consideration of this the diagnosis was changed from Hodgkin's sarcoma to benign lipoma or a cyst of the mediastinum of undetermined type. The patient was discharged on September 21.

He was not seen again until he was admitted to the hospital on July 8, 1938 in a moribund condition. He died on July 9 before further examination could be made.

Autopsy.—The body was that of a thin white boy 14 years old. There were no palpable lymph nodes. There was no edema. The veins of the chest were prominent.

When the thoracic cavity was opened, a huge mass was seen filling the mediastinum, completely replacing the thymus and extending down to a short

distance above the diaphragm. From side to side it measured 22 cm. It compressed both lungs, pushing them down. On section the mass was composed of several lobulated portions, and the cut surface was pale pinkish, moist, with large areas replaced by mucin, which was yellow in color. The trachea was pushed to the right, and the esophagus was compressed by the mass.

The pericardial cavity was pushed down. It contained a normal quantity of serous fluid.

The heart weighed 350 Gm. A slight bulging could be seen about the middle of the anterior wall of the left ventricle. In the apex the bulge was slightly paler than the rest of the heart. The right side of the heart was normal and contained only postmortem clots. The left auricle was normal. A large yellowish polypoid tumor filled the cavity of the left ventricle, growing from the anterior wall of the myocardium. It measured 5.5 cm. in diameter. The base of the tumor involved the anterior wall and the interventricular septum of the left ventricle and extended to the anterior cusp of the mitral valve but did not involve it. The remaining findings were irrelevant.

Fig. 2 (case 1).—Section of the tumor and adjacent heart muscle. Hematoxylin and eosin stain; × 120.

Microscopic Examination.—The mediastinal mass was unusual, with a somewhat varying picture in different parts. The striking feature was the areas made up of ribbon-like bands of cells, branching occasionally and showing longitudinal striations. The cytoplasm was acidophilic, and the nuclei were centrally located. The nuclei were oval, with a reticular chromatin network and a very small nucleolus. In some areas the cells became narrow, with fine elongated nuclei, resembling plain muscle. In other parts there was a loose mucoid stroma and the cells were long and spidery but with plump nuclei. There were many thin-walled blood vessels. The tumor of the heart presented a picture resembling that of the mediastinal tumor, with the edges similar to the less differentiated areas of that tumor.

The mediastinal lymph node showed all the features of the mediastinal tumor mass.

The node in the left intercostal muscle disclosed tumor similar to the mediastinal tumor.

Other microscopic alterations were not significant.

Fat stains of the tumor were negative for the presence of fat.

Diagnosis.—Rhabdomyosarcoma of the heart metastasizing to the mediastinal lymph nodes, the pleura and the intercostal muscle.

CASE 2.—*Rhabdomyoma of the Heart.*—This tumor was encountered in a 6½ month old stillborn fetus. The mother, 29 years old and white, had the Rh factor in her red cells, group A. She was admitted to the hospital on Nov. 11, 1946 as threatened with abortion. Her last normal menstrual period was stated to have been June 20. She had been regularly attending the prenatal clinic from August 1946 to October 1946. On November 11 she awakened during the night to find herself lying in a pool of blood. She called a doctor, who immediately had her admitted to the hospital. On admission the uterus was 2 fingerbreadths above the umbilicus and there were active fetal movements. On November 12 her temperature was elevated, and penicillin therapy was begun, with resulting improve-

Fig. 3 (case 2).—Cut surface of the heart, showing the tumor attached to the left ventricle. In the wall of the ventricle are several smaller nodules.

ment and fall of temperature. On November 15 the membranes ruptured spontaneously and the fetus was expelled, followed one hour later by the placenta. The fetus was dead on delivery.

After delivery, the patient's illness ran a septic course. She left the hospital against medical advice on November 21.

Gross Examination of the Fetus.—The fetus was female, measuring 24 cm. from crown to rump and 34 cm. from crown to heel, and weighed 820 Gm. The approximate age according to measurements was 26 weeks (Potter and Adair [13]). The skin of the legs was discolored bluish red, and the dorsal surfaces of both feet were edematous. To the umbilicus was attached 10 cm. of umbilical cord, measuring 1.5 cm. in diameter. The heart weighed 7.8 Gm.; the surface vessels were congested, and there were numerous subpericardial petechial hemorrhages.

---

13. Potter, E. L., and Adair, F. L.: Fetal and Neonatal Death, ed. 1, Chicago, University of Chicago Press, 1940, pp. 23-26.

Located on the left side and attached to the left ventricle was a circumscribed tumor mass, measuring 1.4 by 1.3 by 1.0 cm. The lateral surface was convex, and the medial surface was flattened where it had been applied to the wall of the ventricle. The external surface was gray, and the cut surface was grayish white, smooth and homogeneous. Scattered in the myocardium were several other whitish tumor nodules, measuring as much as 3 mm. in diameter.

The remaining findings were not significant.

Microscopic Examination.—The largest tumor of the myocardium was located beneath the pericardium. Scattered throughout the wall of the left ventricle were numerous well circumscribed tumors. One of these was present in a papillary muscle immediately beneath the endocardium. The tumor cells varied considerably

Fig. 4 (case 2).—*A*, photomicrograph of the margin of the largest tumor showing the cytologic structure. Normal myocardium is seen in the lower right corner. Hematoxylin and eosin; × 180. *B*, photomicrograph of a giant spider cell with a centrally located nucleus and radiating cytoplasmic strands separated by vacuoles. Geschickter stain; × 200.

in size, from 10 to 50 microns. The cytoplasm was vacuolated and was granular and netlike, and in areas definite cross striations were seen. The cells did not contain fat. (Staining for glycogen, unfortunately, could not be done, because of incorrect fixation of the tissues.)

Microscopic examination of the remaining fetal parts showed nothing of significance except a large subcapsular hemorrhage of the liver.

The placenta measured 15 by 10 by 3 cm. and weighed 410 Gm. To the fetal surface was attached 25 cm. of umbilical cord. The material surface showed a large part of one pole to be replaced by blood clot which infiltrated throughout the entire thickness of the placenta. The membranes attached to the placenta appeared adequate. Microscopic examination showed the placenta to be well vascularized, with an area of recent hemorrhage.

Diagnosis.—Congenital rhabdomyoma of the heart.

SUMMARY

Two cases of primary tumor of the heart are presented, one a case of rhabdomyosarcoma occurring in a 14 year old boy, the other a case of rhabdomyoma or congenital nodular glycogenic tumor of the heart as an incidental finding in a 26 week fetus.

Notes and News

Appointments, Etc.—A. J. Canny, former senior pathologist and acting director of the Kanematsu Institute of Pathology, Sydney Hospital, has been appointed to the chair of pathology at the University of Queensland, in Australia.

Harry Eagle, of the Johns Hopkins University School of Hygiene and Public Health, has been appointed scientific director of the National Cancer Institute. He will have charge of the extensive program of research within the institute.

Charles S. Cameron has been appointed acting medical and scientific director of the American Cancer Society, following the resignation of Dr. Ashley W. Oughterson.

Hamilton R. Fishback, associate professor of pathology at Northwestern University Medical School and pathologist and director of laboratories at Norwegian-American Hospital, Chicago, has resigned to accept the position of pathologist and director of laboratories at Herrick Memorial Hospital, Berkeley, Calif.

In Brazil, Amadeu Fialho, pathologist of the National Department of Health, has been appointed professor of pathology at the University of Rio de Janeiro, succeeding Paul Leitao da Cunha, who died a few weeks ago.

R. H. Rigdon, formerly professor of pathology at the University of Arkansas School of Medicine has been appointed professor of experimental pathology in the University of Texas, Galveston.

M. D. Eaton, director of the research laboratory of the state department of health at the University of California, has been appointed associate professor of bacteriology and immunology in the Harvard Medical School, Boston.

William D. McNally, toxicologist of the coroner's office of Chicago for twenty-one years, has resigned.

The retirement of S. B. Wolbach as professor of pathology in the Harvard Medical School has been announced. He has accepted the directorship of nutritional research at the Children's Hospital, Boston.

A. C. Ivy, vice president of the University of Illinois, has been appointed executive director of the National Advisory Cancer Council, succeeding George M. Smith, professor emeritus of anatomy in Yale University, who resigned for reasons of health.

Death.—Clement C. Fenton, professor of pathology in the medical school of the University of West Virginia, Morgantown, died May 24, 1947, at the age of 54, from coronary occlusion.

Medicolegal Conference and Seminar.—The departments of legal medicine of the medical schools of Harvard, Tufts and Boston universities, in association with the Massachusetts Medico-Legal Society, will present a six day program (Oct. 13 to 18 1947) of lectures, conferences and demonstrations having to do with the investigation of deaths in the interests of public safety. Attendance will be limited to 25 persons who have registered in advance. Further information may be obtained from the department of Legal Medicine, 25 Shattuck Street, Boston.

Examination for Certification in Pathology.—The American Board of Pathology has declared that after Jan. 1, 1948 all applicants for certification will be required to take the examination of the board. The last meeting of the American Board of Pathology before this date will be from October 24 to 25 in Chicago.

Books Received

Biochemistry of Cancer. By Jesse P. Greenstein, Ph.D., head biochemist and chairman of Section on Biochemistry, National Cancer Institute, National Institute of Health, United States Public Health Service, Bethesda, Md. Cloth. Pp. 389, with 39 illustrations. Price $7.80. New York: Academic Press, Inc., 1947.

The announcement of a new book on the biochemistry of cancer is greeted with enthusiasm in the hope that the extensive recent work in this field will be well summarized and correlated. In a large measure this anticipation is justified. The author is well qualified by a number of years of cancer research to write the book.

The monograph is divided into three sections dealing respectively with the induction of tumors, attempts at control of the induction and the growth of tumors, and the properties of tumors. The first section is subdivided into chapters on extrinsic and intrinsic factors; the second has chapters on nutrition, endocrinologic factors and chemotherapy; the third deals with the chemistry of tumors and of the tumor-bearing host. The monograph thus contains a broader coverage of subject matter than would be expected from the title. On the other hand, little space is given to some chemical subjects, such as immunity.

The third section is the best. It summarizes a large amount of material from many sources. In other sections the presentation of factual material is, on the whole, more impressive than are the generalizations. The important role of chemical studies, as one among many others, in solving problems of the nature and the causation of cancer is repeatedly emphasized. The bibliographies are incomplete but large and useful, nevertheless. While there is no question of accuracy of statements of fact there is sometimes question regarding the best possible shading of emphasis, owing at least in part to lack of inclusiveness which, as the author states, has not been attempted. Despite this lack the book should be useful to all who are concerned with cancer. It is not only an excellent and readable review of the biochemistry of cancer but a good introduction to many other aspects of cancer research. It can be highly recommended to students. Busy pathologists who want a review and reference book should find it valuable.

The Incidence of Neurosis Among Factory Workers. By Russell Fraser, with the collaboration of Elizabeth Bunbury, Barbara Daniell, M. Elizabeth Barling, F. Estelle Waldron, P. Mary Kemp and Imogen Lee. Medical Research Council, Industrial Health Research Board Report no. 90. Pp. 65. Price 45 cents. London: His Majesty's Stationery Office, 1947.

Neuropathology in Its Clinicopathologic Aspects. By I. Mark Scheinker, M.D., assistant professor of medicine (neurology) and instructor in neuropathology, University of Cincinnati College of Medicine; neuropathologist and attending neurologist, Cincinnati General Hospital. With a Foreword by Tracy J. Putnam, M.D., professor of neurology and neurological surgery, College of Physicians and Surgeons, Columbia University, and director of services of neurology and neurologic surgery, Neurological Institute of New York. Pp. 306, with 209 illustrations. Price $6.75. Springfield, Ill.: Charles C Thomas, Publisher, 1947.

A Textbook on Pathology of Labor, the Puerperium and the Newborn. By Charles O. McCormick, M.D., clinical professor of obstetrics, Indiana University School of Medicine; consulting obstetrician to William H. Coleman Hospital for Women, Indianapolis City Hospital and Sunny Side Sanitarium. Second edition. Pp. 514, with 272 illustrations, including 24 in color. St. Louis: C. V. Mosby Company, 1947.

MULTIPLE MYELOMA

A Survey Based on Thirty-Five Cases, Eighteen of Which Came to Autopsy

LOUIS LICHTENSTEIN, M.D.

AND

HENRY L. JAFFE, M.D.

NEW YORK

MULTIPLE MYELOMA (myelomatosis) is a clinically and anatomically distinctive malignant disease of the skeleton which apparently takes its departure from the myeloid formative tissue proper. The disease is by no means rare. It seems probable that many cases go unrecognized. The condition is more common in males than in females, but not strikingly so. It shows a predilection for persons who are between 40 and 60 years of age, and though it is not unusual in those who are in the 30's, it is definitely uncommon in persons who are less than 30 years of age.

Anatomically, practically every bone may ultimately come to be involved, more or less, in a given case. The progress of the disease may be steady and rapid, sometimes from the beginning and sometimes after a static period. One gathers also that in some cases, before the disease becomes spread over the skeleton, it may flourish in one bone (as a so-called solitary myeloma) for months or even years. Though at autopsy the skeleton may be found riddled through with foci of myeloma, it is only infrequently that gross myelomatous foci are found in the viscera and other extraskeletal parts. Nevertheless, even in the absence of gross infiltrations, microscopic examination sometimes reveals the presence of smaller or larger numbers of myeloma cells within the spleen, the liver or the lymph nodes, and occasionally in other organs as well. Also, in some cases myeloma cells invade the blood stream. Ordinarily, under these circumstances, relatively few myeloma cells are found in the blood smears. However, in an occasional case they may be so numerous as to create a leukemic blood picture (so-called plasma cell leukemia).

This discussion of multiple myeloma is based on 35 cases, in all but 3 of which the diagnosis of myeloma was confirmed by examination of tissue. In the 3 excepted cases, although Bence Jones proteinemia was found in only 1, the diagnosis seemed fairly certain from the fact that

---

From the Laboratory Division, Hospital for Joint Diseases.

hyperglobulinemia or hypercalcemia or both were in association with roentgenographic skeletal changes consistent with multiple myeloma. In the great majority of our cases the roentgenographic examination was not limited to the skeletal region giving clinical difficulties but, indeed, covered a substantial part of the skeleton, so that the full extent and distribution of the tumor process was mirrored. In more than half the cases, particularly in those observed in recent years, chemical estimations of serum albumin and globulin and of serum calcium were made. In all but a few of the cases the urine was examined for Bence Jones protein, often repeatedly. Eighteen of the 35 patients came to autopsy. The examination of these 18 included a detailed study of the skeleton. Specifically, much of the vertebral column, the sternum, many of the ribs, parts or all of the pelvic bones and, in many instances, some of the long bones and the calvarium were removed and cut open. Furthermore, in many instances the sectioned bones were then roentgenographed, and in all instances tissue was removed from many representative areas and studied microscopically.

CLINICAL CONSIDERATIONS

Age and Sex Incidence.—Fully three fourths of the patients were between 40 and 60 years of age. This finding is in accord with the generally accepted view that multiple myeloma shows a predilection for persons who are in later middle life. The incidence of the disease appears to fall off sharply in persons who are past 60. As to its incidence in persons less than 40 years of age, as noted, it is by no means unusual in those who are in the 30's but is rather rare in those who are less than 30. Only 1 of our patients was below this age, the one in question being a boy of 17, who presented the first manifestations of the disease at the age of 13. A few unequivocal instances of multiple myeloma occurring in adolescents or children have also been reported.[1] Some others, less convincing, in the literature may actually be instances of Schüller-Christian disease, stem cell leukemia, Ewing's sarcoma or neuroblastoma that has metastasized to the skeleton.

As noted, our data indicate that multiple myeloma may be slightly more prevalent in males than in females but do not support the often repeated statement that the condition is at least twice as frequent in males.

Clinical Complaints.—Evaluation of the clinical records of our cases showed that the major difficulties (occurring singly or in combination) which brought the patients to the hospital were, in the order of decreasing frequency: (1) pain, especially of the back and the thorax, (2) substan-

1. Gordon, H., and Schneider, B.: Internat. Clin. **4**:173, 1940. Berkheiser, E. J.: Arch. Surg. **8**:853, 1924. Laurentius, P.: Monatschr. f. Kinderh. **73**:95, 1938.

tial loss of weight, (3) pathologic fracture of some bone and (4) a palpable tumor appearing in relation to a superficial flat bone. It should be pointed out, however, that patients seeking admission to our hospital tend to do so mainly because of complaints directly referable to skeletal alterations rather than because of anemia, abnormal bleeding, neurologic symptoms or findings suggesting nephritis.

The pain occurring in the back and the thorax was often vague and generalized, and accompanied by a feeling of weakness. However, in the presence of compressed or collapsed vertebral bodies it had a more localized, persistent and disabling character and was frequently associated with some manifestations of compression of the spinal cord or at least of issuing nerve roots. The loss of weight amounted to 30 pounds (13.5 Kg.) or even more in some cases. As the disease progressed, there was a tendency, even in those who showed no appreciable loss of weight on admission, toward increasing debilitation and terminal emaciation. Pathologic fracture as expressed in compression collapse of one or more vertebral bodies was not uncommon, but in some cases a femur or a humerus, for instance, presented a pathologic fracture extending through an area of exuberant myelomatous involvement. If one or more tumor masses are palpable on the skeleton, they are most likely to be found in relation to a clavicle or to one or several ribs. However, a tumor may sometimes be palpable in relation to such superficial bone sites as the calvarium or the facial bones, the sternum, a scapula or an innominate bone.

Presenting Skeletal Lesions.—There are some cases of multiple myeloma in which the patients complain of rather generalized bone pain without specific localization, in spite of widespread skeletal involvement. Usually, however, it is difficulty with some particular bone or skeletal region that first directs attention to the presence of the disease. The involved site most often responsible for difficulties in those whose initial skeletal complaints were localized was the vertebral column. Indeed, in approximately one half of these patients the difficulties were referable to the spine. The lumbar region was most often affected, next the dorsal and least often the cervical region. Patients having complaints referable to the vertebral column often showed roentgenographic evidence of compression or collapse of one or more vertebral bodies on admission to the hospital, and a number of them presented signs of resulting compression of the cord as well (fig. 3 *C*). It seems worth mentioning that, with involvement of the lumbar region of the spine, disability and sciatic pain were common complaints even in the absence of compressed or collapsed vertebral bodies.

The long bones, particularly the femur, represented another clinically important site of localization. Specifically, there were 5 patients each of whom presented in a femur a strikingly large focus of myeloma that

rendered the area easily susceptible to pathologic fracture. This large focus of destruction was found sometimes in the midshaft and sometimes at the end of the shaft. Roentgenographically, the femoral lesions were regularly misinterpreted as representing something other than multiple myeloma until their true nature was established by biopsy (fig. 8 *D*). Large, destructive lesions comparable to those observed in femurs were also observed occasionally in humeri.

When it occurs in a superficially located bone, the presenting lesion is also likely to be clinically palpable. Thus, not infrequently, such a
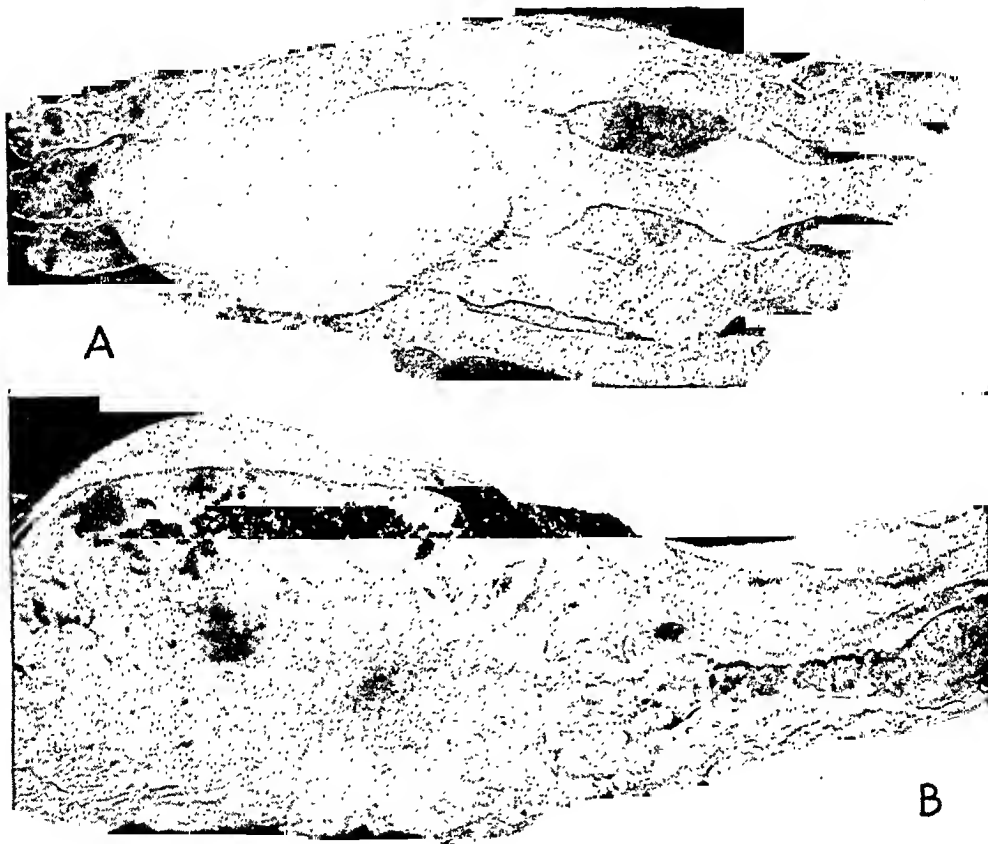
Fig. 1.—*A*, roentgenogram (reduced) of myeloma involving several ribs in a case of far advanced multiple myeloma in which an autopsy was made. A large parosteal tumor mass is seen where the myeloma has extended through the cortex of one of the ribs. The ribs are extremely porotic, expanded, and honeycombed by tumor tissue. This subject was known to have had multiple myeloma for at least nine years.

B, photograph of the expanded tumor shown in *A*. The tumor tissue is hemorrhagically mottled in places. The pleura serves as an effective restraining barrier, but on the external surface the neoplastic tissue has extended beyond the periosteum into the adjacent muscle and connective tissue.

tumor can be felt on a rib or a clavicle (fig. 1). Actually, however, even a bone of a foot or a hand may sometimes be the site of the presenting lesion.

Clinical Course and Prognosis.—In many cases the disease pursues an insidious course in the beginning, the patient complaining merely of some weakness, loss of weight or vague pain in the back or the chest. Sometimes, a fracture of some bone (commonly a rib or a limb bone) weakened by an exuberant focus of tumor within it is the first major difficulty. In other instances the disease may be ushered in dramatically by sudden onset of severe root pain or even paraplegia resulting from compression or collapse of one or more vertebral bodies. Taking our cases altogether, we find that the duration of symptoms prior to hospitalization ranged from as short a time as a few weeks to as long as two years, the average duration being about nine months.

Whatever the initial complaints that bring the patients to the hospital, the progress of skeletal involvement thereafter, as judged by successive roentgenograms, is rather variable and frequently unpredictable. The patients who present a far advanced stage of the disease when first observed usually (though not invariably) survive no more than some weeks or months. As for those with still limited involvement roentgenographically, in some one observes tremendous progression within just a few months, while from others one gains the impression that the neoplasm progresses relatively slowly or remains static for months or even a number of years before entering its terminal phase. This variability of tempo is not explainable on a cytologic basis alone. The average period of survival following the onset of symptoms was about two years in our cases, which is in accord with the general impression, but there are striking deviations from this average. Two patients in whom transverse myelitis developed succumbed within one and three months, respectively, although it is true that neither of them received the possible benefit of laminectomy for decompression. On the other hand, we did an autopsy on a patient whose history of the disease dated back over nine years, and another patient (presenting tumor in an ilium initially) is still alive and comparatively well after ten years. While these instances are exceptional, it is well known that the clinical course of multiple myeloma may occasionally be protracted and characterized by long remissions, which are frequently attributed to roentgen treatment but which may be spontaneous. Cases in point have been reported by Gross and Vaughn,[2] Kirsch,[3] Batts[4] and Davison and Balser.[5] The latter cited an extraordinary case which came to autopsy sixteen years after the onset of symptoms. It is true also that patients in whom the tumor is apparently localized in some one bone in the beginning not infre-

2. Gross, R. E., and Vaughn, W. W.: Am. J. Roentgenol. **39**:344, 1938.
3. Kirsch, I. E.: M. Bull. Vet. Admin. **18**:96, 1941.
4. Batts, M., Jr.: Arch. Surg. **39**:807, 1939.
5. Davison, C., and Balser, B. H.: Arch. Surg. **35**:913 and 935, 1937.

quently go on for a number of years before the myeloma becomes disseminated over the skeleton.[6]

In most cases, as the disease progresses there is a tendency toward demineralization and devastation of the skeleton, associated with increasing anemia and cachexia, provided the clinical course is not cut short, as it frequently is, by such complications as intercurrent infection (especially pneumonia in bedridden patients), concomitant cancer of some other kind, cardiac failure, renal insufficiency, ascending infection of the urinary tract and amyloidosis.

PROBLEMS OF DIAGNOSIS

There are numerous cues to the diagnosis of multiple myeloma coming from many quarters—clinical, roentgenographic, hematologic, biochemical —and the recognition of the condition prior to biopsy or postmortem examination frequently requires their fullest utilization. It is true that the diagnosis of multiple myeloma will be fairly obvious if many bones, including the calvarium, are veritably riddled by osteolytic defects (the picture usually stressed in the texts) and if, in addition, Bence Jones proteinuria is discovered. Unfortunately, this skeletal picture represents the exception rather than the rule, in our experience at least, and Bence Jones proteinuria is as likely to be absent as present. Indeed, often one observes merely some vaguely defined rarefactions in some of the bones or a single exuberant tumor in a single bone without obvious involvement of the skeleton generally, and sometimes (when myelomatous infiltration of the bone marrow is diffuse) skeletal changes may not be apparent at all roentgenographically in spite of complaints referable to the skeleton. In such equivocal or initially obscure cases the lead may come from the discovery of anemia, the presence of myeloma cells in blood smears, hypercalcemia, hyperproteinemia (and certain peculiar hematologic manifestations resulting from increase of serum globulins), evidences of renal damage of a peculiar type or even from the finding of unusual tumor-like amyloid deposits. It must be recognized, however, that these pertinent findings are not all present in every case or necessarily present in the early stages of the evolution of any given case, nor are they necessarily pathognomonic in themselves. It is only by utilizing all the logical approaches that one is likely to arrive at a combination of significant findings constituting probable or conclusive evidence of the presence of multiple myeloma.

For anatomic confirmation, puncture of the sternal marrow should be freely employed. A high degree of reliability is claimed for this procedure, though there are undoubtedly cases of multiple myeloma in

---

6. (a) Bailey, C. O.: Am. J. Roentgenol. **36**:980, 1936. (b) Pasternack, J. G., and Waugh, R. L.: Ann. Surg. **110**:427, 1939. (c) Stewart, M. J., and Taylor, A. L.: J. Path. & Bact. **35**:541, 1932.

which sternal marrow spreads fail to yield significant information. Biopsy of some obviously affected and readily accessible bone will resolve any possible doubt as to the diagnosis, since the histologic recognition of a myeloma entails no difficult problems in differential diagnosis as a rule.

SKELETAL ALTERATIONS AND THEIR ROENTGENOLOGIC INTERPRETATION

No single description can do justice to the gross appearance of the bones in all cases of multiple myeloma coming to autopsy. At one extreme there is the occasional case in which the bones appear normal as to surface and contour and those removed do not even offer any striking lack of resistance when being cut open. On inspection of the cut surfaces of these bones, one finds that the spongy trabeculae are still numerous and that the cortices are not significantly thinned. However, the marrow is modified and replaced more or less diffusely by rather whitish tissue which on histologic examination is proved to be myeloma tissue. In conformity with these findings, the skeletal roentgenograms taken during life in such a case may show at most some diffuse porosity of the bones. They certainly show nothing even remotely suggestive of the roentgenographic picture one has been taught to regard as representative of multiple myeloma. This was the skeletal status in a case of atypical amyloidosis and myeloma which we studied. It reemphasizes the fact that in every case of atypical amyloidosis, despite the lack of evidence of multiple myeloma in the clinical roentgenograms, bones must be opened and their marrow examined histologically to establish or rule out the presence of myeloma.

In another occasional case, while the bones show smooth and undistended contours, they cut with abnormal ease. When cut, such bones show thinning of the cortices from the medullary side, as well as great reduction of the spongy trabeculae. This is the result of encroachment on the osseous tissue by a gray-whitish tissue which has substantially replaced the marrow and which stands out in some places as discrete foci of tumor. The clinical roentgenograms of the bones in such a case hardly suggest the conventional idea of the roentgenographic picture of multiple myeloma but do reflect the tumor encroachment on the osseous tissue by showing vague mottled or vacuolated rarefactions and thinned cortices.

In many cases, however, the cortex becomes gravely weakened or even destroyed by the tumor tissue in one or several places of one or even of many bones. Thus, there is a bulge in the contour of the bone at such a site, and the tumor tissue which has spread out of the bone is found distending the periosteum but still restrained by it; or having violated the periosteum, it may even be found invading the local musculature. Exuberant growth of the myeloma at one bone site, with destruction of the cortex and spread of the tumor beyond the bone in this area, often

produces the lesion which first calls attention to the disease, though the marrow of the skeleton as a whole may already be riddled through by tumor tissue. For instance, a patient with multiple myeloma may first present himself because of a localized palpable and often painful enlargement of a rib, a clavicle, a jaw bone or even a long bone of an extremity —particularly a femur or a humerus. The patient showing such a lesion in a femur or a humerus may already have a pathologic fracture at the site of the presenting lesion (figs. 1 and 8).

However, it is remarkable how often one finds multiple myeloma presenting itself clinically because an exuberant focus of the disease has developed in a vertebral body (or several contiguous bodies). The affected body or bodies are found substantially destroyed; indeed, the tumor tends to transgress them, often producing pressure on the spinal cord or the local nerve trunks, with symptoms resulting (fig. 3 C). In such cases roentgenographic examination of the rest of the skeleton (including the skull) often fails to reveal the clearcut punched-out rarefactions which are conventionally held to distinguish the roentgenographic picture of multiple myeloma. It is in such cases (especially if one dorsal or lumbar body alone is clearly affected) that the true nature of the disease often goes unrecognized for long periods, the lesion frequently being interpreted as a local one, i.e., as a hemangioma, a giant cell tumor, a fracture due to Kümmell's disease or, if grossly destructive, as a focus of metastatic cancer (fig. 8 B and C). The true nature of the disease may become clear if marrow is obtained by sternal puncture or if serial roentgenograms of the rest of the skeleton finally demonstrate widespread lesions in other bones suggesting multiple myeloma, but sometimes it is discovered only at autopsy. Eventually, in the far advanced stage of the disease, the bones of the trunk and the limbs may become so extensively porotic and deformed from riddling by neoplastic tissue as to present the appearance of washed-out honeycomb bed shells roentgenographically. However, this extreme expression of skeletal devastation is observed only occasionally, even at autopsy (figs. 1 A and 2 A, B and C).

A somewhat special interest attaches to the calvarium. Great emphasis is usually laid on the diagnostic value of several or even many roundish punched-out rarefactions revealed in roentgenograms of the skull. It is true that when roentgenograms show clearcut and widespread involvement of the rest of the skeleton in the form of numerous punched-out rarefactions, the calvarium, too, is quite likely to show these, though not infrequently it fails to display them. But it is precisely when one turns to the roentgenograms of the calvarium because those of the other bones do not show the conventional picture of multiple myeloma that the calvarium, too, fails to show it. Whether or not the calvarium shows rarefactions, histologic examination will reveal that the marrow of the

Fig. 2.—Roentgenograms of bone specimens obtained at autopsy illustrating the skeletal devastation that may be observed in far advanced multiple myeloma: *A*, an iliac bone showing a washed-out, honeycombed appearance, reflecting extreme porosity of the spongy bone and thinning of the cortex. *B*, vertebral column (same case) showing striking resorption of bone and compression of many of the vertebral bodies. *C*, several involved ribs (same case). *D*, a slice of the calvarium (a comparable case) showing a sharply punched-out defect in the tables. The involvement of the calvarium was considerably less pronounced than the involvement of the skeleton generally.

diploic spaces has been substantially replaced by the tumor tissue. At sites of clearcut rarefaction one will find that the tumor tissue is present as a nodule which has encroached on and destroyed the diploic bone, sometimes also thinning the tables, but in our experience the tables are seldom perforated even in such sites (fig. 7). Actually, the riddling of
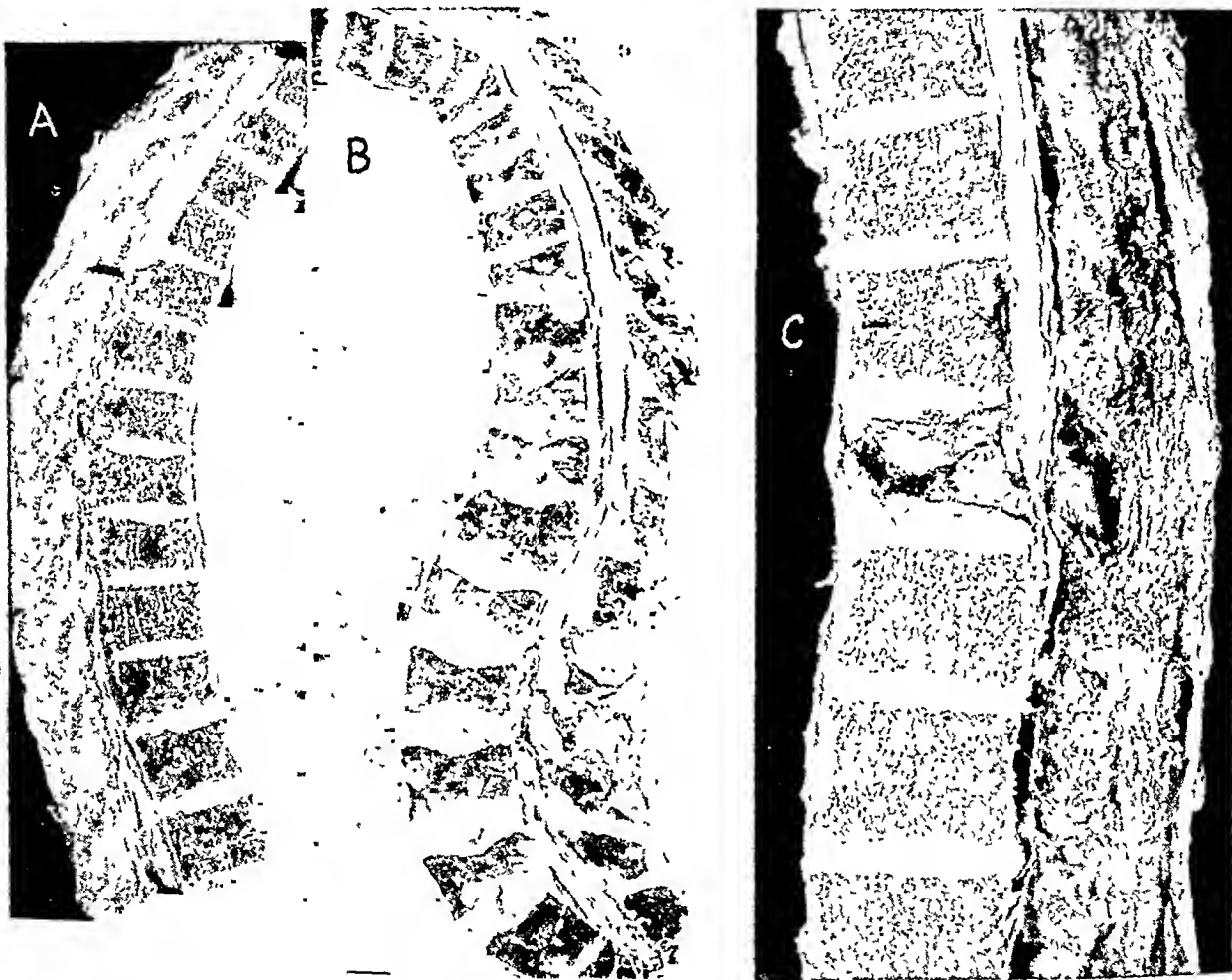
Fig. 3.—Specimens from several autopsies illustrating variations in the degree and the type of involvement of the vertebral column. *A* shows such inconspicuous lesions grossly that one has to search for scattered small nodes of myeloma tissue within the marrow of some of the vertebral bodies. *B* displays striking involvement. The vertebral bodies are extremely porotic and compressed; many of the intervertebral disks are correspondingly expanded; the marrow everywhere has been extensively replaced by myeloma, which also appears in the form of discrete tumor nodes; there are also tumor nodes of varying size within some of the spinous processes. The roentgenogram of this spine is pictured in figure 2 *B*. The skeletal changes in this case were associated with pronounced hypercalcemia and profound anemia. *C* shows almost total destruction and collapse of the body of the tenth dorsal vertebra and also extradural tumor encuffment of the cord at that level. The other vertebrae were not collapsed but showed miliary to small pea–sized grayish white areas of tumor tissue, some bodies presenting more of these than others. The compression of the spinal cord was followed by transverse myelitis and ascending infection of the urinary tract. This subject (a woman of 35) presented a leukemic blood picture terminally.

the calvarium by tumor deposits, which produce numerous circular punched-out defects, is not invariably an indication of multiple myeloma. since it sometimes occurs in carcinomatosis.[7]

HEMATOLOGIC OBSERVATIONS OF DIAGNOSTIC IMPORTANCE

In our cases the hematologic findings were essentially in agreement with those recorded in the literature, which point to the significant frequency with which anemia is encountered with multiple myeloma. The anemia reflects progressive neoplastic encroachment on the myeloid marrow, bleeding into tumor tissue and frequently also the effects of

Fig. 4.—Macerated specimen of the lower lumbar vertebral bodies and the innominate bones, showing moth-eaten erosions of these bones in places invaded by myeloma tissue.

general debilitation and renal damage. About 70 per cent of our patients presented appreciable reduction of hemoglobin and erythrocyte levels when first observed, and in about one half of them the anemia was already of moderate or severe grade. For example, a patient with far advanced skeletal involvement had a hemoglobin value on admission of only 4.2 Gm. per hundred cubic centimeters of blood and a red blood cell count of 1,400,000 per cubic millimeter. In any event, even if the anemia is not profound at first, the trend in most cases followed over a period of

7. Cosin, L.: Brit. J. Surg. **23**:110, 1935.

several or many months is toward slow but steady decline of the hemoglobin and erythrocyte values, often to appallingly low levels. Transfusions, even when repeated, appear to have merely a temporary sparing effect. In a notable case under observation for approximately a year, the hemoglobin value fell as low as 2.6 Gm. and the erythrocyte count to 1,000,0000 and remained at about these levels for six months until death resulted from cachexia, renal insufficiency and terminal bronchopneumonia. The profound anemia was reflected anatomically in intense hemosiderosis of liver, spleen, marrow and lymph nodes. Incidentally, the blood smears showed as many as 20 to 30 normoblasts per hundred white cells counted. The anemia of this particular patient was normocytic, as anemia usually is in cases of myeloma,[8] the color index being slightly below 1.0, but the anemia of some myelomatous patients may be macrocytic in type, simulating pernicious anemia.[9]

The depletion and irritation of the leukopoietic marrow in cases of myeloma is not infrequently reflected by the appearance in the blood smears of a few myelocytes or even myeloblasts, and occasionally these immature leukocytes may be present in such large numbers as to produce a leukemoid picture.[10] Thus, multiple myeloma, from a hematologic point of view at least, sometimes simulates myeloid leukemia at first, especially if a purpuric tendency is present. Sometimes, also, the number of eosinophils may be found increased as another indication of irritation of the marrow. The total leukocyte count shows no consistency and may be normal, somewhat depressed or slightly increased.

It is pertinent to mention also that any of the following phenomena may be observed in cases of multiple myeloma: excessive rouleau formation in blood smears, autohemagglutination of the red cells in dry and wet films, clumping with Hayem's solution, failure of clot retraction, abnormal viscosity of the blood and rapid sedimentation rate. It has been shown also that serums from myelomatous patients often have an anticomplementary property, although by no means all such serums give this reaction.[11] These phenomena in general have been ascribed to the presence of hyperproteinemia (which we shall discuss farther on), and their discovery may afford the first cue to the diagnosis of multiple myeloma.[12]

8. Wintrobe, M. M.:   Clinical Hematology, Philadelphia, Lea & Febiger, 1942, p. 631.

9. Sweigert, C. F.:   Am. J. M. Sc. **190**:245, 1935. Astrua, P. C.:   Minerva med. **1**:358, 1939.

10. Krumbhaar, E. B.:   Am. J. M. Sc. **172**:519, 1926.

11. Jersild, M.:   J. A. M. A. **113**:1119, 1939.

12. Foord, A. G.:   Ann. Int. Med. **8**:1071, 1935. Foord, A. G., and Randall, L.: Am. J. Clin. Path. **5**:532, 1935. Jersild.[11]

Another important aid in the diagnosis of myeloma through hematologic methods is the finding of myeloma cells or so-called atypical plasma cells in blood smears. The frequency with which such cells are discovered and properly identified (beyond their provisional designation as "blast" cells) seems to depend largely on the thoroughness and skill of the observer,[13] and in suspected cases of myeloma blood smears should really be scrutinized by a qualified hematologist. They were discovered in only 2 of our cases on routine examination,[14] but Morissette and Watkins[15] claimed to have found them in as many as 41 of 56 cases studied. They pointed out that preliminary scrutiny of the smear under low magnification was necessary for the detection of these abnormal cells.

In most instances in which they are found, the myeloma cells are present in relatively small or, at most, moderate numbers, but occasionally there may be such an outpouring of them into the blood stream as to give rise to a frankly leukemic blood picture. Thus, in 1 of our cases (in which transverse myelitis was the cause of death) the leukocyte count during the terminal phase of the disease rose steadily from 6,000 to 40,000, and 30 to 54 per cent of the leukocytes were identified as plasma cells. Such cases of multiple myeloma in which the tumor cells readily invade the blood stream are well known and have been collected and discussed under the head of plasma cell leukemia by Muller and McNaughton,[16] Piney and Riach,[17] Osgood and Hunter,[18] Patek and Castle[19] and others. The pertinent cases coming to autopsy indicate conclusively that so-called plasma cell leukemia has its anatomic foundation in skeletal multiple myeloma and cannot logically be considered, as Osgood and Hunter have suggested, as a disease primarily of the blood. The development of "leukemia" in such cases represents a phase, and often a terminal phase, in the evolution of the disease. It is worth noting that cases of multiple myeloma characterized by frank invasion of the blood stream apparently exhibit a strong tendency toward extraskeletal spread in the form of diffuse and occasionally nodular infiltrations of the spleen, the liver and lymph nodes, and sometimes also of the kidney, the pancreas, the skin or other organs.

Still another valuable diagnostic aid has been advanced in recent years by hematologists, namely, the use of sternal marrow puncture. The

13. Mallory, T. B.: New England J. Med. **212**:28, 1935.

14. Rubin, G.: Bull. Hosp. Joint Dis. **3**:62, 1942.

15. Morissette, L., and Watkins, C. H.: Proc. Staff Meet., Mayo Clin. **17**: 433, 1942.

16. Muller, G.-L., and McNaughton, E.: Folia haemat. **46**:17, 1931.

17. Piney, A., and Riach, J. S.: Folia haemat. **46**:37, 1931.

18. Osgood, E. E., and Hunter, W. C.: Folia haemat. **52**:369, 1934.

19. Patek, A. J., and Castle, W. B.: Am. J. M. Sc. **191**:788, 1936.

experience of Rosenthal and Vogel,[20] Beizer and co-workers,[21] Waldenström[22] and others indicates that sternal puncture has high diagnostic value in cases of multiple myeloma, although it may not necessarily yield positive information in every instance.[22a] Certainly, it is a procedure that should be employed whenever the presence of myeloma is suspected, and especially when biopsy of some obviously affected bone is not feasible. In most instances of multiple myeloma, according to Wintrobe,[8] cellular marrow spreads will reveal myeloma cells making up 3 to 65 per cent of the total number of cells.

SIGNIFICANT BIOCHEMICAL CHANGES IN CASES OF MULTIPLE MYELOMA

Hypercalcemia.—The occurrence of hypercalcemia with multiple myeloma was noted [23] as early as 1927, while the observation that hypercalcuria and a negative calcium balance may be present dates back thirty years or more.[24] Hypercalcemia occurs in about one half of the cases of multiple myeloma, having been observed by us in 8 of the 16 cases investigated (in the remainder the calcium values were within the normal range). The increased calcium values ranged as high as 18 mg. per hundred cubic centimeters of serum. It should be noted that in such cases the kidneys tend to show deposits of calcium granules in the tubular epithelium and interstitial connective tissue. In some cases the metastatic calcifications may be quite heavy and widespread, involving, in addition, the interstitium of the lungs, the lining of the stomach and even other tissues.[25]

The increase of calcium in the serum reflects the lytic resorption of the bones and the tendency toward renal failure in many of the cases. It apparently develops independently of hyperproteinemia, since the highest calcium levels which we observed were in cases in which the serum protein concentration was normal. Apparently, in some instances the tendency toward hypercalcemia is perpetuated and accentuated by secondary hyperplasia of the parathyroid glands developing in response to chronic renal insufficiency. However, this mechanism does not invariably operate as Bulger and his co-workers believe.[26] Indeed, in our own

20. Rosenthal, N., and Vogel, P.: J. Mt. Sinai Hosp. **4**:1001, 1938.

21. Beizer, L. H.; Hall, B. E., and Giffin, H. Z.: Am. J. M. Sc. **203**:829, 1942.

22. Waldenström, J.: Acta chir. Scandinav. **87**:365, 1942.

22a. Aegerter, E., and Robbins, R.: Am. J. M. Sc. **213**:282, 1947.

23. Charlton, T. J.: Arch. Int. Med. **40**:98, 1927.

24. Williams, O. T.: Biochem. J. **5**:225, 1911. Blatherwick, N. R.: Am. J. M. Sc. **151**:432, 1916.

25. (*a*) Bender, O.: Deutsche Ztschr. f. Chir. **63**:370, 1902. (*b*) Froboese, C.: Virchows Arch. f. path. Anat. **222**:291, 1916.

26. Bulger, A. A.; Dixon, H. H.; Barr, D. P., and Schregardus, O.: J. Clin. Investigation **19**:143, 1930.

autopsies in which the parathyroid glands could be dissected out, these glands did not show significant enlargement.

Hypercalcemia per se is, to be sure, not diagnostic of multiple myeloma even when it is associated with rarefying skeletal lesions, since it also occurs characteristically with idiopathic hyperparathyroidism and occasionally with osteoclastic carcinoma extensively metastasizing to the skeleton. However, hypercalcemia associated with either hyperproteinemia or Bence Jones proteinuria is clearly indicative of multiple myeloma. It is precisely because of the presence of hypercalcemia, nephrocalcinosis and resorptive skeletal changes that some cases of multiple myeloma have been misinterpreted, at least temporarily, as instances of idiopathic hyperparathyroidism[27] and the patients even subjected to surgical exploration in a misguided search for a parathyroid adenoma.[28] Actually, as Jaffe[29] has pointed out, a proper appreciation of the roentgenographic appearance of the calvarium in cases of multiple myeloma should suffice to prevent confusion with hyperparathyroidism, irrespective of the roentgenographic changes elsewhere.

Furthermore, multiple myeloma is characterized by the fact that the serum phosphatase activity tends to be normal, no matter how extensive the skeletal involvement may be. It is true that if the serum phosphatase activity is measured shortly after the occurrence of a pathologic fracture, it may be found slightly increased, but the increase does not attain the level that is usually reached in advanced stages of hyperparathyroidism. In doubtful cases, in which the skeletal lesions are equivocal roentgenographically, the serum protein values are not significantly elevated and Bence Jones proteinuria is not detectable, marrow obtained by sternal puncture should also help to resolve the problem in differential diagnosis.

Hyperproteinemia.—One of the peculiar and characteristic features of some cases of myeloma is the presence of hyperproteinemia, specifically hyperglobulinemia. Indeed, the occurrence of abnormal protein in the blood of patients with multiple myeloma was noted as early as 1899 by Ellinger.[30] Hyperglobulinemia is observed in about half the cases of multiple myeloma; it was present in 8 of 16 cases investigated by us. Indeed, its incidence is claimed by some to run as high as 60 per cent. The globulin values as determined by the conventional Howe method in these cases ranged between 3 and 14 Gm. per hundred cubic centimeters of blood. The formaldehyde-gel and Takata reactions provide

27. Caylor, H. D., and Nickel, A. C.: Ann. Surg. **97**:823, 1933. Enzer, N., and Lieberman, B.: Ann. Int. Med. **8**:1062, 1935. Sager, W. W.; Choisser, R. M., and Weller, G. L., Jr.: J. Lab. & Clin. Med. **23**:1132, 1938.

28. North, J. P.: Am. J. Surg. **31**:563, 1936.

29. Jaffe, H. L.: Bull. New York Acad. Med. **16**:291, 1940.

30. Ellinger, A.: Deutsches Arch. f. klin. Med. **62**:254, 1899.

convenient crude tests for hyperglobulinemic serums,[31] but when positive they should be supplemented by precise quantitative methods. The serum albumin does not contribute to the hyperproteinemia, being normal, as a rule, when the globulin value is normal and actually diminished when the globulin value is increased. Thus, in cases of our series in which the serum globulins were normal in concentration the albumin values ranged between 4 and 5 Gm., while in 8 cases in which the globulin (and total protein) values were increased, the albumin values ranged between 2 and 3.5 Gm. The reason why the serum albumin values are consistently low in the cases of myeloma characterized by hyperglobulinemia seems to be that, in these cases particularly, damage of the renal tubules tends to develop, and thus the loss of albumin may conceivably be part of the complex of renal insufficiency.

Hyperglobulinemia per se is, of course, not necessarily indicative of multiple myeloma, since it is known to occur with other conditions also—particularly with chronic infections, notably lymphogranuloma venereum, sarcoidosis and kala-azar, and occasionally with cirrhosis of the liver, chronic nephritis, etc. However, hyperglobulinemia should always suggest the possibility of myeloma, and if found in association with hypercalcemia or Bence Jones proteinuria, speaks definitely for myeloma.

The protein composition of myeloma serums has been the subject of active investigation in recent years. Magnus-Levy,[32] who has written extensively on multiple myeloma, has advanced the view that the hyperproteinemia reflects exclusively an increase of the euglobulin and that the latter accounts for most of the physical aberrations of the blood commonly observed in cases of myeloma. We found the euglobulin significantly increased in only 3 of the 6 hyperglobulinemic serums investigated. In these 3 serums the high "euglobulin" ranged from 5 to 11 Gm. per hundred cubic centimeters and constituted 70 to 80 per cent of the total globulin, thus accounting for most, if not all, of the protein increment. However, in the other 3 serums the euglobulin levels were within the normal range.

Actually, Kekwick,[33] Devine [34] and Gutman, Moore, Kabat and their co-workers,[35] employing new physicochemical technics, have shown that the problems entailed in the separation, identification and quantitative estimation of the various normal and abnormal proteins which may be

31. Bing, J.: Acta med. Scandinav. **91**:336, 1937. Gutman, A. B., and Wise, C. R.: Proc. Soc. Exper. Biol. & Med. **35**:124, 1936. Waldenström.[22]

32. Magnus-Levy, A.: Ztschr. f. klin. Med. **126**:62, 1933.

33. Kekwick, R. A.: Biochem. J. **34**:1248, 1940.

34. Devine, J.: Biochem. J. **35**:433, 1941.

35. Gutman, A. B.; Moore, D. H.; Gutman, E. B.; McClellan, V., and Kabat, E. A.: J. Clin. Investigation **20**:765, 1941. Moore, D. H.; Kabat, E. A., and Gutman, A. B.: ibid. **22**:67, 1943.

present in the serums of myelomatous patients are extremely complex. The last-mentioned authors, in particular, have shown that there is extraordinary variability of the protein increment as determined not only by conventional fractional precipitation with neutral salts but also by improved electrophoretic technic (Tiselius apparatus), ultracentrifugation, protein solubility curves and immunologic methods. They have found that, apparently, the serums of most of the patients fall into one of three classifications: (1) serums of apparently normal composition with respect to serum proteins, (2) serums with hyperglobulinemia due to increase chiefly in the Howe euglobulin fraction and partly in the pseudoglobulin I fraction, comparable to what one may observe in chronic infections, and (3) serums with or without hyperglobulinemia giving a variety of anomalous patterns by Howe or electrophoretic methods, not encountered in other diseases, and apparently due for the most part to significant Bence Jones proteinemia. Their observations have led them to believe, furthermore, that Bence Jones protein, so called, is not a single substance but an ill defined group of similar proteins, thus confirming the observations of Bayne-Jones and Wright [36] based on immunologic reactions of Bence Jones proteins.[37]

36. Bayne-Jones, S., and Wright, W. D.: Bull. Johns Hopkins Hosp. **33**:37, 1922.

37. The origin of these abnormal serum proteins, particularly of the Bence Jones proteins, has been the subject of much discussion. There is evidence at hand to indicate that serum proteins other than albumin and fibrinogen (which are believed to be synthesized in the liver) may originate in leukocytic cells of lymphoid or bone marrow origin. For example, gamma globulins concerned with antibody formation are believel to be derived from lymphoid cells (Dougherty, T. F.; Chase, J. H., and White, Proc. Soc. Exper. Biol. & Med. **57**:295, 1944; Kass, E. H.: Science **101**:337, 1945). More pertinent are the experiments of L. Meyler (Arch. Int. Med. **57**:708, 1936) on the nature of a Bence Jones-like protein substance extractible from normal marrow and also from leukocytes obtained from pus and leukemic tissue. These experiments suggest that small undetectable amounts of Bence Jones (or similar) protein normally derive from the sources indicated and that in multiple myeloma (and rarely also in certain other conditions affecting the marrow) the output is so magnified above the rate of destruction that the protein appears in the blood and is excreted as a pathologic constituent. Thus, there seems to be some justification for holding that the myeloma cells themselves, being derived presumably from leukopoietic marrow tissue, may elaborate and store abnormal proteins, and indeed the presence of a Bence Jones-like protein in myelomatous tissue has been demonstrated. On the other hand, there are those [34] who contend that the considerable amount of protein excreted in some cases of myeloma precludes the possibility of the protein's being a metabolite of the tumor cells and who hold that the protein in question results from a derangement of a synthetic mechanism in the marrow, which may result also in the formation of excessive euglobulin and of certain abnormal proteins, and possibly of amyloid substances as well (Boggs and Guthrie [43]; Magnus-Levy [32]).

This multiplicity of Bence Jones proteins and the difficulty of separating them from other normal and abnormal serum proteins make further progress in their identification and assay perplexing, although for the detection of small amounts of Bence Jones protein in serum the specific precipitin reaction devised by Hektoen [38] has been employed to advantage. Nor does the complexity of the problem end there. The observations of Wintrobe and Buell,[39] von Bonsdorff, Groth and Packalén [40] and Shapiro, Ross and Moore [41] indicate that the serums of myelomatous patients may on occasion contain still another peculiar abnormal protein substance of high molecular weight and great viscosity, differing from Bence Jones protein and showing a tendency to spontaneous precipitation and crystallization.

Bence Jones Proteinuria.—As is well known, the occurrence in the urine of protein giving the Bence Jones reaction is the earliest recorded observation in connection with the neoplasm now commonly designated multiple myeloma, dating back a century. It is also generally recognized that the presence of this reaction strongly favors the diagnosis of multiple myeloma (with certain reservations) but that, on the other hand, it may be absent in established cases of myeloma. We found evidence of Bence Jones protein in the urine in 10 of 26 cases investigated, although it has been stated [42] that it is found in at least 65 per cent of all cases of multiple myeloma. This discrepancy may be more apparent than real, and one must be persistent in searching for Bence Jones protein in the urine, since: (1) it may be undetectable in casual specimens but present in a twenty-four hour specimen, (2) it may be present at certain times but not at others (that is, its excretion may be intermittent rather than continuous) and (3) it may be absent early in the course of the disease and become evident later on. On the other hand, there are undoubtedly instances of myeloma in which no detectable Bence Jones protein is ever excreted. In 1 of our cases twenty-five examinations for Bence Jones proteinuria were made over a period of a year, all of them yielding negative results, including that made on urine taken from the bladder at autopsy. We have not observed any absolute correlation between the cytologic aspects of our myelomas and the incidence of Bence Jones proteinuria, although we do have the impression that the latter occurs somewhat more frequently with the relatively large cell myelomas

38. Hektoen, L.: J. A. M. A. **76**:929, 1921.

39. Wintrobe, M. M., and Buell, M. V.: Bull. Johns Hopkins Hosp. **52**:156, 1933.

40. von Bonsdorff, B.; Groth, H., and Packalén, T.: Folia haemat. **59**:184, 1938.

41. Shapiro, S.; Ross, V., and Moore, D. H.: J. Clin. Investigation **22**:137, 1943.

42. (*a*) Geschickter, C. F., and Copeland, M. M.: Arch. Surg. **16**:807, 1928. (*b*) Wintrobe and Buell.[39]

(characterized by hyperglobulinemia) than it does with the classic small cell plasmacytoma.

In regard to the specificity of Bence Jones proteinuria, it is universally stated that Bence Jones protein may occasionally be found in the urine in certain conditions other than multiple myeloma, especially in leukemia and in carcinoma metastatic to the skeleton. However, citations of actual experience to that effect are scarce in the literature. We have never encountered it in other conditions ourselves, although we have not made any systematic study of the Bence Jones reaction in skeletal diseases other than myeloma. There are a few pertinent observations, however—namely, those of Boggs and Guthrie,[43] Geschickter and Copeland [42a] and Bayrd and Heck [44]—purporting to show that rarely in chronic leukemia and metastatic carcinoma of bone and still more rarely in certain other diseases of bone (e. g., senile osteomalacia, gunshot wound, polycythemia) the urine may contain protein giving the Bence Jones reaction. With these exceptions, the finding of Bence Jones proteinuria is, for all practical purposes, indicative of multiple myeloma.

Increased Uric Acid in the Blood.—This represents still another significant, though not specific, change occurring with multiple myeloma. It has been commented on by Stewart and Weber [45] and has been observed by Tarr and Ferris [46] and also by us in several instances. In the opinion of Stewart and Weber, the uric acid increment results from the catabolism of nucleoproteins derived from the myeloma cells—an explanation that seems quite plausible. Multiple myeloma is, of course, not the only tumor responsible for hyperuricemia; it is well known, for example, that the latter may also occur with the leukemias.

EXTRASKELETAL MYELOMATOUS INFILTRATIONS

As noted, the extraskeletal occurrence of grossly discernible myelomatous foci is decidedly uncommon, although there can be no doubt that in occasional cases of multiple myeloma coming to autopsy (particularly in those in which the blood stream had been invaded by myeloma cells) one may find single or even multiple tumor foci within the internal organs. Microscopic infiltrations, particularly of the spleen, the liver or lymph nodes, are somewhat less unusual, but in our experience even these are lacking in most cases of multiple myeloma. Not infrequently, what may appear to be independent extraskeletal tumor foci occurring, for example, in the dura, the pituitary gland, the oropharynx and the nasopharynx, the larynx, the thyroid gland, the pleura and the retroperitoneal, mediastinal and subcutaneous tissues may actually represent direct outgrowths

43. Boggs, T. R., and Guthrie, C. G.: Bull. Johns Hopkins Hosp. **23**:353, 1912.
44. Bayrd, E. D., and Heck, F. J.: J. A. M. A. **133**:147, 1947.
45. Stewart, A., and Weber, F. P.: Quart. J. Med. **7**:211, 1938.
46. Tarr, L., and Ferris, H. W.: Arch. Int. Med. **64**:820, 1939.

of tumor of contiguous bones. Also, in evaluating the reported cases of supposed multiple myeloma characterized by extensive and widespread tumor deposits in the internal organs, it is important to bear in mind that some of them may actually have been instances of metastasizing occult carcinoma,[47] malignant lymphoma or stem cell leukemia.

Nevertheless, cases of myeloma demonstrating the presence of extraskeletal tumor foci are prominently featured in the literature precisely because of their unusual nature, and infiltration of practically any organ that might be named has been noted at one time or another. Thus, Mallory [48] has cited a case of multiple myeloma in which a single metastatic nodule was found in a lung (and also in a bronchial lymph node), and similar instances of pulmonary metastasis have been noted also by Hallermann,[49] Carlisle,[50] Piney and Riach [17] and Batts.[4] Metastases have been observed in the heart by Piney and Riach and by Carlisle, who commented on the finding of two discrete bean-sized nodules of myeloma within the wall of the right auricle. Nodular infiltrations of an enlarged spleen have been observed by Osgood and Hunter,[18] and Churg and Gordon [51] have described a remarkable case in which the spleen (weighing 650 Gm.) presented innumerable tumor nodules studding and substantially replacing the pulp. In the latter case, infiltration of some of the intra-abdominal lymph nodes and of the portal areas and sinusoids of the liver was also noted. Piney and Riach have described the finding of a tumor node, the size of a plum, within the pancreas, and Patek and Castle,[19] with regard to a case exhibiting "plasma cell leukemia," commented on the observation of a soft, reddish brown tumor node, 1.5 cm. in diameter, in the tail of the pancreas. In the latter case, incidentally, infiltration of the liver, the spleen, the kidneys and the abdominal lymph nodes was also noted microscopically. A metastasis in the form of a cherry-sized tumor node has been observed in an adrenal gland by Piney and Riach, and adrenal metastases have been noted by others. Involvement of the kidney has been noted by Carlisle, Morse,[52] Donhauser and DeRouville [53] and by Newns and Edwards,[54] the last describing the finding of an essentially circumscribed rounded tumor, 4 cm. in diameter, replacing part of the lower pole of a kidney and bulging into its pelvis. Involvement of the tonsils was noted in an extraordinary case reported by Jackson, Parker and

47. Nicholls, A. G.: Canad. M. A. J. **17**:301, 1927.
48. Mallory, T. B.: New England J. Med. **215**:1133, 1936.
49. Hallermann, W.: Deutsches Arch. f. klin. Med. **165**:57, 1929.
50. Carlisle, V.: South African M. J. **12**:298, 1938.
51. Churg, J., and Gordon, A. J.: Arch. Path. **34**:546, 1942.
52. Morse, P. F.: J. Cancer Research **5**:345, 1920.
53. Donhauser, J. L., and DeRouville, W. H.: Arch. Surg. **43**:946, 1941.
54. Newns, G. R., and Edwards, J. L.: J. Path. & Bact. **56**:259, 1944.

Bethea,[55] in which a "plasmacytoma" in that region had been removed fully eight years before generalized involvement of bone could be detected, although the neoplasm had extended to the cervical lymph nodes in the interim. Striking involvement of the intestinal tract has been described by Carlisle,[50] who found numerous tumor deposits in the submucosa of the duodenum and small bowel, the mucosa being tautly stretched over these protuberances. Ovarian metastases have been noted by Herrick and Hektoen,[56] and bilateral tumorous involvement of the male gonads has been observed by Ulrich.[57] Cutaneous involvement in the form of multiple raised nodular infiltrations (scattered over the scalp, the region of the clavicle and the arm) has been described by Duvoir and associates,[58] and Kin [59] has reported a comparable case in which the nodular infiltrations (over the face and the back) were large, umbilicated and ulcerated, presenting the general picture of so-called mycosis fungoides. Piney and Riach [17] have described a most unusual case of multiple myeloma with "leukemia" in which the subject presented a diffuse nodular eruption over the trunk and limbs, looking not unlike neurofibromatosis clinically, and also a peculiar, helmet-like thickening extending down from the infiltrated scalp of the region of the forehead, in front of the ears and down to the nape of the neck. Incidentally, in this case, as in some of the others characterized by a leukemic blood picture, histologic examination showed infiltration of multiple sites, including the cervical lymph nodes, the kidney, the liver and the heart.

In our own autopsy material we encountered extraskeletal tumor foci only twice. In one instance (in which myeloma cells had been detected in blood smears during life) we encountered a solitary circumscribed whitish nodular focus of myeloma, about 0.5 cm. in diameter, within the liver subcapsularly. In this case we also observed collections of myeloma cells within the sinuses of the spleen on microscopic examination. In the other case, the lymph nodes along the aorta, within the mesentery, at the hilus of the liver and that of the spleen and around the pancreas were all more or less distinctly enlarged by sheets of tumor cells replacing the normal structure. The liver and the spleen were likewise appreciably enlarged and were seen microscopically to be infiltrated by collections of similar cells. Indeed, the possibility of aleukemic lymphadenosis was at first considered despite a positive Bence Jones reaction, but the observation that the bone marrow was diffusely

55. Jackson, H., Jr.; Parker, F., Jr., and Bethea, J. M.: Am. J. M. Sc. **181:** 169, 1931.

56. Herrick, J. B., and Hektoen, L.: M. News **65:**239, 1894.

57. Ulrich, H.: Arch. Int. Med. **64:**994, 1939.

58. Duvoir, M.; Pollet, L.; Layani, F.; Dechaune, M., and Gaultier, M.: Bull. et mém. Soc. méd. d. hôp. de Paris **54:**687, 1938.

59. Kin, S. S.: Arch. f. jap. Chir. **16:**79, 1939.

infiltrated by cells resembling myeloma cells and the finding of renal changes characteristic of multiple myeloma established the latter diagnosis. unequivocally.

Whether myeloma cell infiltrations of the liver, the spleen or lymph nodes represent hematogenous metastases of tumor occurring in the bones or, rather, tumor foci independently formed as a result of activation and neoplasia of potential blood-forming cells within the organs in question does not concern us here directly. Sometimes infiltration of the spleen and the liver may already be manifested grossly. by whitish streaks or nodules, or in the case of lymph nodes, by diffuse enlargement and cellularity, but more often it is detected only on microscopic examination. On the other hand, our experience definitely runs counter to that of Lowenhaupt,[60] who claimed to have found "plasma cells" of myelomatous nature in every one of the spleens from 12 patients who came to autopsy, in all of the lymph nodes examined and in three of the livers as well. It should be pointed out, however, that the indubitable identification of isolated small nests of cells presumed to be myeloma cells and their clearcut differentiation from cells of histiocytic or inflammatory nature commonly encountered in the spleen and the lymph nodes, particularly, may be exceedingly difficult. Furthermore, as Mallory[61] has rightly emphasized, small collections of immature blood cells representing foci of compensatory extramedullary hemopoiesis are observed rather frequently in cases of multiple myeloma, and these may also be mistaken for nests of myeloma cells.

SIGNIFICANT RENAL CHANGES IN CASES OF MULTIPLE MYELOMA

In the patient with multiple myeloma there are certain cytologic renal changes of almost pathognomonic distinctiveness. These changes may be manifested clinically in more or less heavy albuminuria, though not necessarily Bence Jones proteinuria, and in diminished power of the kidney to concentrate and to clear nitrogenous constituents. Only exceptionally do they result in the development of edema or hypertension. It has been emphasized on that account (Foord and others) that the finding of "atypical nephritis" should always suggest the possibility of multiple myeloma. When the renal damage is limited in extent, it results clinically in no more than persistent albuminuria. On the other hand, when it is more severe, it often leads ultimately to progressive renal insufficiency. Occasionally it leads to the relatively early appearance of azotemia, which dominates the clinical picture before the presence of multiple myeloma is even suspected.[62]

60. Lowenhaupt, E.: Am. J. Path. **21**:171, 1945.
61. Mallory, T. B.: New England J. Med. **224**:559, 1941.
62. McDonald, R. H.: Cleveland Clin. Quart. **10**:36, 1943. Thannhauser, S. J., and Brereton, H.: Bull. New England M. Center **4**:99, 1942.

Already in 1921, Löhlein [63] noted the deposition of crystalloid hyalin-like casts in the renal tubules of a subject with multiple myeloma coming to autopsy. Many of the casts plugging the tubules were surrounded by polymorphonuclear leukocytes, and often also by giant cells, which Löhlein thought were derived from proliferated tubular epithelium. There was some dilatation of the tubules, also some interstitial scarring, but the glomeruli and the blood vessels were not remarkable. The specificity of these essential changes was confirmed by Perla and Hutner,[64] Ehrich,[65] Bell,[66] Fishberg,[67] Forbus and co-workers,[68] Morison,[69] Blackman and his co-workers,[70] Newns and Edwards [54] and others. Indeed, Mallory [71] and his colleagues spoke of the "myeloma kidney" and maintained that it is often possible to make a diagnosis of multiple myeloma from looking at the kidney sections alone.

Grossly, in our 18 autopsies the kidneys were usually not remarkably altered. In 2 instances, however, they were found to be markedly contracted as a result of extensive interstitial scarring. On reviewing the sections of kidneys obtained in the autopsies we, too, were impressed by the constant finding of rather dense eosinophilic hyalin-like plugs of proteinaceous material in the renal tubules, particularly in the lower portions of the nephrons. In about half of the kidneys examined, one could find foreign body giant cells or clumps of polymorphonuclear leukocytes or both about the casts, although at times one had to search for them (fig. 5). In some instances the proteinaceous plugs were rather scattered, but in others they were numerous. It is pertinent in this connection to recall the exceptional case reported by Holman, in which the tubular obstruction was extensive enough to result in complete urinary suppression. It is noteworthy that the characteristic tubular plugs were found by us in cases in which the Bence Jones reaction had been negative as well as in those in which it had been positive. It seems altogether probable that abnormal proteins other than Bence Jones protein are also precipitated out in the tubules. We observed no significant glomerular changes in our material, although it may be noted in passing that Foord [12] emphasized the possible role of

63. Löhlein, M.: Beitr. z. path. Anat. u. z. allg. Path. **69**:295, 1921.

64. Perla, D., and Hutner, L.: Am. J. Path. **6**:285, 1930.

65. Ehrich, W.: Ztschr. f. klin. Med. **121**:396, 1932.

66. Bell, E. T.: Am. J. Path. **9**:393, 1933.

67. Fishberg, A. M.: Hypertension and Nephritis, ed. 4, Philadelphia, Lea & Febiger, 1939, p. 369.

68. Forbus, W. D.; Perlzweig, W. H.; Parfentjev, I. A., and Burwell, J. C., Jr.: Bull. Johns Hopkins Hosp. **57**:47, 1935.

69. Morison, J. E.: J. Path. & Bact. **53**:403, 1941.

70. Blackman, S. S., Jr.; Barker, W. H.; Buell, M. V., and Davis, B. D.: J. Clin. Investigation **23**:163, 1944.

71. Mallory, T. B.: New England J. Med. **221**:983, 1939.
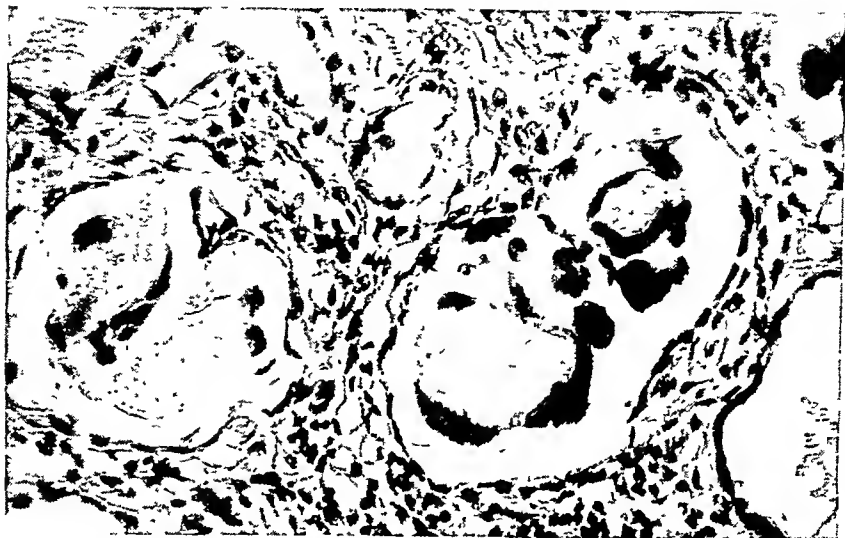
Fig. 5.—Photomicrograph of a section of kidney from a case of multiple myeloma, showing tubules obstructed by proteinaceous casts, about which there is a conspicuous foreign body giant cell reaction. ×200.
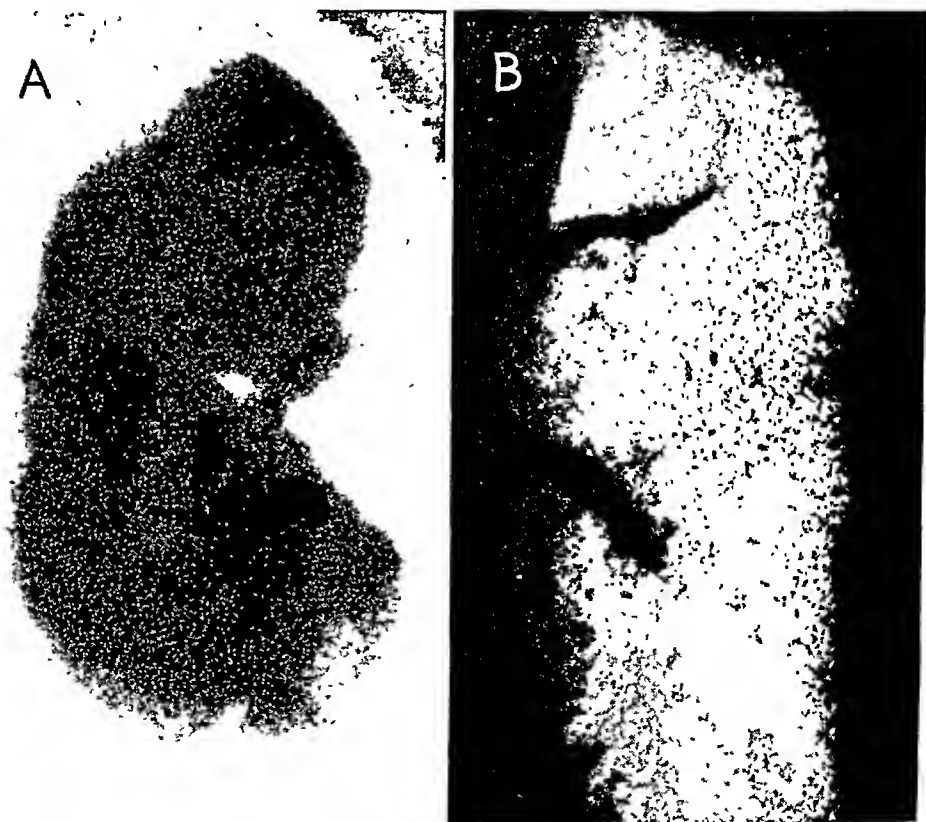
Fig. 6.—Roentgenograms from a case of multiple myeloma (the one pictured in figs. 2 *A, B* and *C* and 3 *B*) illustrating metastatic calcification. *A,* kidney showing calcareous material deposited within some of the calices (calcific gravel was present also in the urinary bladder). *B,* lung showing heavy calcification, principally of the lower lobe. The subject (a man of 32), toward the end of his course, presented extreme demineralization of the skeleton, hypercalcemia (17.8 mg. of calcium per hundred cubic centimeters of serum) and mounting renal insufficiency.

obstruction of glomerular capillaries by highly concentrated protein or by clumped erythrocytes as a factor in the development of renal insufficiency. We did, however, observe degenerative changes in the tubular epithelium in a number of the kidneys, as evidenced by the granular, vacuolar or hyaline droplet appearance of the lining cells and a tendency toward desquamation.

Some of the kidneys also exhibited a number of other changes worthy of mention. In several, slight to moderate arteriosclerotic contraction was noted (which must be evaluated in the light of the fact that these subjects were older adults). In one kidney pyelonephritis was present, having followed on compression of the spinal cord and ascending infection of the urinary tract. In another what appeared to be amyloid in granular or droplet form was present within the tubular epithelium and lumens, though not in the glomeruli. In one (in a case of far advanced myeloma featured by rapidly progressing, extreme demineralization of the skeleton and hypercalcemia, the calcium amounting to almost 18 mg.) evidence of deposition of calcium was observed grossly as well as microscopically (fig. 6 *A*). In this kidney (there was also heavy metastatic calcification of the lungs [fig. 6 *B*]) one could recognize yellowish streaks within some of the pyramids, and fine yellowish calcific gravel was present in many of the calices and within the urinary bladder as well.

AMYLOIDOSIS IN RELATION TO MULTIPLE MYELOMA

Another arresting feature of the pathologic anatomy of multiple myeloma is the presence of amyloid in some cases. Atkinson[72] found amyloidosis complicating myeloma in 40 of 643 cases of myeloma collected from the literature. On this basis the incidence is about 6 per cent. In our own material it was about 10 per cent. The presence of amyloid, particularly since it seems to occur in association with hyperglobulinemia and Bence Jones proteinuria, affords ground for speculation as to the chemical relations which may exist between the abnormal blood proteins and the amyloid protein substances. It has been suggested[73] that the abnormal blood proteins may serve as the mother substance of the amyloid proteins and that the amount and the distribution of the amyloid are determined also by disturbed fibroblastic activity.[74] Whatever the mechanism of amyloid formation may be in the body in general, it does seem probable that excessive formation of abnormal globulins is an essential condition for the deposition of amyloid, and it is conceivable that the latter represents a reaction to the foreign proteins in question.

72. Atkinson, F. R. B.: M. Press **195**:312 and 327, 1937.
73. Magnus-Levy.[32] Tarr and Ferris.[46]
74. Warren, S.: Am. J. Path. **6**:161, 1930.

So far as the skeleton is concerned, if amyloid is found, it usually appears in the form of scattered deposits detectable only microscopically in the neoplastic tissue of the affected bones ('fig. 7). In an occasional case,[75] however, one finds not only the microscopic deposits but large agglomerations of amyloid intermingled with and substantially replacing myelomatous foci. In Freund's [75a] case the amyloid took the form of multiple calcifying amyloid tumors, one of which, breaking out of the seventh dorsal vertebral body, had produced extradural compression of the cord. The presence of multiple myeloma as a basis for the amyloid tumors in this case was recognized only on microscopic examination, there having been no grossly discernible tumor nodes within the bones but rather a diffuse infiltration of the marrow. In this case the amyloid was entirely limited to the skeleton. On the other hand, we have observed a case of so-called atypical amyloidosis in which autopsy failed to reveal evidence of amyloid in the myelomatous tissue itself, though practically
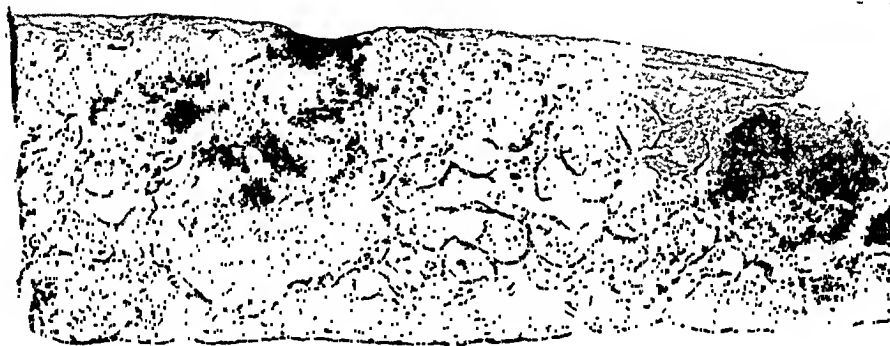
Fig. 7.—Photomicrograph of a cross section of the calvarium from a case of multiple myeloma, showing nodular foci of amyloid intermingled with myeloma cells. The outer table of the calvarium is completely eroded in one area. × 6.

all the extraskeletal tissues and organs, including the voluntary muscles and the skin, were heavily infiltrated with amyloid.

In general, one of the striking features of amyloidosis appearing in association with multiple myeloma is the frequency with which amyloid is deposited in unusual sites, either more or less diffusely or in the form of tumor-like masses which may attain large bulk. In contrast to the commonplace amyloidosis of parenchymatous organs (particularly the liver, the spleen, the adrenal glands and the kidneys), one may find amyloid deposited in great quantity in bones, in muscles, in joint capsules and in the skeletal connective tissues generally, in the skin and subcutaneous tissue, in the buccal and anal mucous membranes, in the tongue, in the heart, in the lungs, in the intestines, in the genitourinary tract

75. (a) Freund, E.: Frankfurt. Ztschr. f. Path. 40:400, 1930. (b) Stadler, L.: Folia haemat. 61:353, 1939. (c) Rosenblum, A. H., and Kirshbaum, J. D.: J. A. M. A. 106:988, 1936.

and in other tissues. Indeed, so often is multiple myeloma the basis for so-called atypical or idiopathic amyloidosis that the possiblity of myeloma should be investigated in every case, even though the bones present no apparent evidence of tumor either roentgenographically or on gross inspection at autopsy.

These cases of unusual amyloidosis are all of great interest, but we may single out certain ones for special comment. In 1 of our cases (the one mentioned on page 28) involvement of the skin and subcutis led to remarkable scleroderma-like thickening and the occurrence of amyloid-containing verrucae, which were found on the eyelids, about the anus, on the oral mucous membrane and along the margins of the enlarged, rubbery, ulcerated tongue. Also noteworthy are the cases in which the mucosa or the muscular coat of the small intestine is extensively infiltrated by amyloid, since this deposition may lead at times to clinically puzzling intestinal obstructon.[76] Of particular interest, also, are the cases standing out because of the presence of multiple, smaller or larger, localized amyloid masses attached to the periosteum of the bones, especially near their articulations, and situated also in the skeletal muscles of the trunk and the extremities and about and within joint capsules. In relation to the latter, the amyloid may extend to the synovium and sublining tissue and, at times, even erupt into the joints.[77] Among the regions that may be selected are the hands, the antecubital fossae, the shoulders and the articulations of the clavicle. The presence of such joint swellings, associated with pain and limitation of motion, sometimes leads to a clinical diagnosis of rheumatoid arthritis, which may be entertained for years before the nature of the condition is recognized.[46] The amyloid masses seen in these cases are described as having a firm, grayish yellow or pinkish, lardaceous or glassy appearance somewhat suggestive of the flesh of fish, and microscopically they present as amorphous, poorly cellular, generally eosinophilic aggregates, about which one may observe foreign body giant cells. It is noteworthy also that this amyloid material, either in part or throughout, may fail to give the usual metachromatic staining reactions with one or another, or perhaps all, of the dyes commony employed for the detection of amyloid.

RELATIONSHIP OF APPARENTLY SOLITARY MYELOMA AND MULTIPLE MYELOMA

As has been stated, there are occasional cases of myeloma in which the first skeletal manifestation is that of an exuberant tumor focus within some one bone (commonly a femur or a humerus, but sometimes a vertebral body, an innominate bone, a bone of the calvarium or some other bone) and in which clearcut roentgenographic evidence of impli-

76. Randall, O. S.: Am. J. Cancer **19**:838, 1933. Bell.[60]

77. Hueter, C.: Beitr. z. path. Anat. u. z. allg. Path. **49**:100, 1910. Paige, B.: Am. J. Path. **7**:691, 1931. Stewart and Weber.[45] Stadler.[75b] Tarr and Ferris.[46]

cation of other bones does not appear for a number of years. We have observed 2 such cases. In one of them, attention was at first centered on an apparently solitary neoplastic focus in the third lumbar vertebral body (fig. 8 *B* and *C*). Indeed, this lesion was irradiated as a possible hemangioma or giant cell tumor. It was not until two and a half years after the onset of symptoms that a veritable shower of foci of myeloma appeared throughout the skeleton. In the other case the presenting focus (identified by biopsy as a myeloma) was a large tumor apparently localized within the upper end of the right femur. This tumor had transformed the upper part of the shaft, the intertrochanteric region and the neck into a ballooned-out, rarefied and coarsely honeycombed lesion simulating a peculiar cyst or giant cell tumor (fig. 8 *D*). Roentgenograms of the pelvis and the upper ends of the femurs (the only roentgenograms taken at the time) showed, otherwise, merely equivocal involvement of the right ilium and suggestive rarefaction shadows within the greater trochanter region of the left femur. This patient received radiation therapy at another hospital, with clinical improvement resulting. The case was subsequently included among those reported by Coley,[78] who likewise interpreted it as one of solitary myeloma. From him we learned that the patient died after a terminal wasting illness suggesting dissemination of tumor, but not until she had survived almost ten years after the onset of complaints referable to the tumor of the femur. Many other cases of supposedly solitary myeloma have been reported, including some with entirely inadequate follow-up records; they attracted attention [79] because they tended to controvert the conventionally doleful prognosis for myeloma in general. Among the more detailed and informative of these reports are those of Bailey,[6a] Pasternack and Waugh [6b] and Stewart and Taylor,[6c] citing survivals of seven, seven and a half and eight years, respectively, in patients who were still alive and apparently in good health at the time of publication. The consensus [79b] with regard to treatment seems to be that roentgen therapy has considerable value in affording relief from pain and possibly also in retarding local growth and eventual dissemination of the tumor.

In regard to these initially localized myelomas, it is hardly possible to determine from the available evidence whether the tumor in any given instance was entirely confined to the skeletal focus first attracting attention or whether it was already present elsewhere in the skeleton but clinically and roentgenographically silent. It has been claimed by the advocates of the former view that the negative result of roentgenographic examination of the remainder of the skeleton affords proof of the solitary nature of the myeloma in question. However, it is well known that the marrow throughout the skeleton may be extensively

78. Coley, W. B.: Ann. Surg. **93**:77, 1931.

79. (*a*) Cutler, M.; Buschke, F., and Cantril, S. T.: Surg., Gynec. & Obst. **62**:918, 1936. (*b*) Gootnick, L. T.: Radiology **45**:385, 1945.
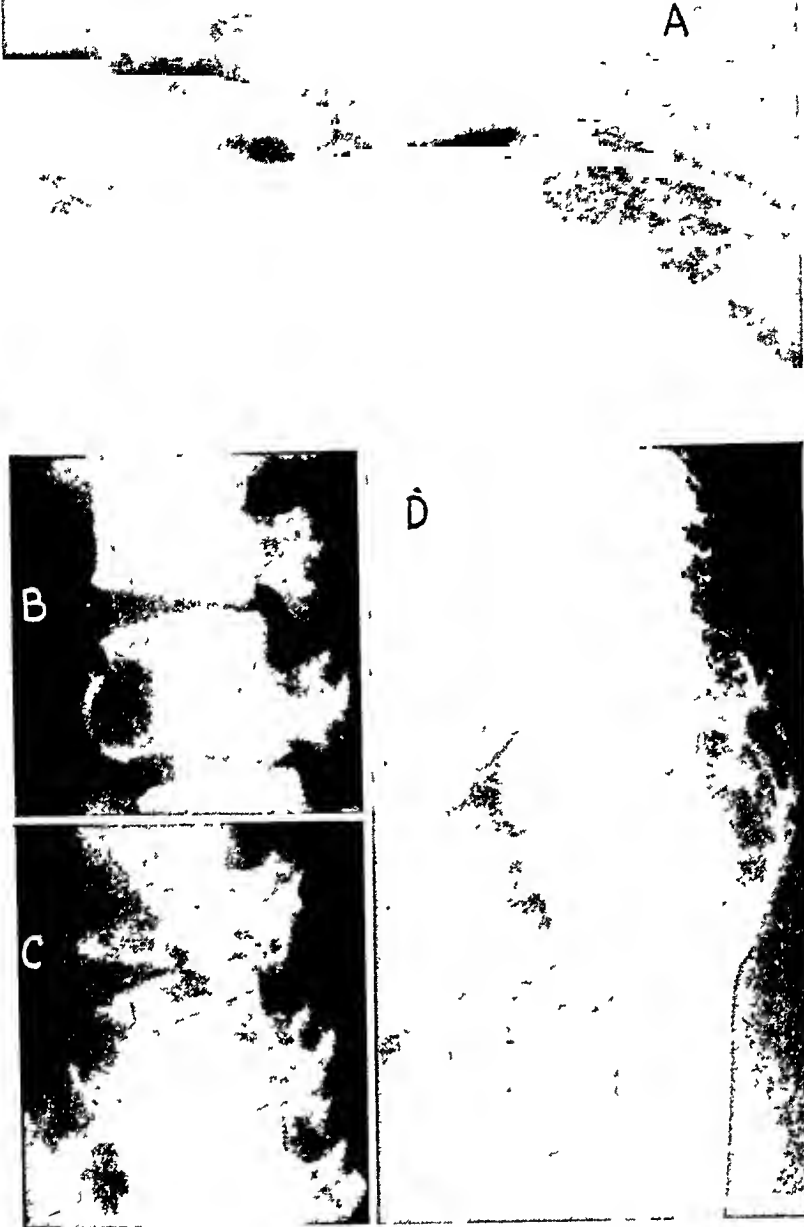
Fig. 8.—Roentgenograms of a number of myelomas from cases in which the tumor appeared initially to be localized in some one bone but subsequently showed obvious spread over the skeleton. Such tumors are usually not recognized as myelomas prior to biopsy, being commonly mistaken for giant cell tumor, hemangioma, bone cyst and other types of growth.

A, a myeloma of the acromial end of a clavicle, which presents an expanded multilocular appearance A fracture extends through the sternal extremity of the expanded area. At the time no clearcut foci of myeloma were discernible elsewhere, but roentgenographic examination almost three years later revealed the presence of a single small punched-out defect in the calvarium and two additional foci in the femurs Also, marrow obtained by puncture of the sternum showed nests of myeloma cells interspersed among the normal constituents of the marrow.

B, a myeloma appearing initially in the third lumbar vertebral body and apparently confined to that bone.

C, the same lesion as it appeared subsequently; the affected body has collapsed in spite of radiation therapy. About two and a half years after the onset of symptoms a shower of myelomatous foci appeared throughout the skeleton.

D, another large, curiously expanded myeloma apparently localized within the upper end of a femur. In this case, also, there was eventual dissemination of the tumor, but the subject survived almost ten years after the onset of complaints.

permeated by myeloma without this being evident roentgenographically. The long period of latency is also cited as proof, but, as we have seen, there is no certainty, when dealing with such a tumor, that foci of . myeloma may not appear throughout the skeleton at any time. Indeed, as such cases are followed the number of survivors falls off steadily from year to year, so that at the end of a ten year period of observation few ostensibly solitary myelomas are left.[79] Also cited as proof of actual one bone localization is the absence of anemia and of Bence Jones proteinuria, but these are hardly trustworthy indications.

A more valid criterion would be sternal marrow punctates showing absence of myeloma cells—a test which was resorted to in few of the cases held to represent cases of genuine solitary myeloma [80]—but even this is not infallible. When one turns to the pertinent autopsy reports in the literature for more satisfactory proof of the existence of genuine solitary myeloma, one finds that there have been few in which the skeleton was examined with sufficient thoroughness both grossly and microscopically to exclude convincingly the possibility that myeloma occurred in other bones. It is true that on the basis of cases such as those reported by Harding and Kimball [81] and by Rutishauser [82] one has to admit the possibility of there being a genuine solitary myeloma, but the condition, if it actually occurs, must be quite rare. All one can say with certainty is that there are occasional cases in which myeloma starts out by producing an exuberant tumor focus in some one bone, commonly a long bone, and then tends to remain latent (in spite of pathologic fractures) for a long time, sometimes even as long as ten years, without giving rise to clearly discernible foci in other bones. The great likelihood is that in such cases the myeloma, if followed for a sufficiently long time, will eventually show obvious dissemination.

CYTOLOGIC CHARACTER OF MULTIPLE MYELOMA

The tumor tissue in multiple myeloma (when its appearance is not modified by hemorrhage, degeneration and necrosis, fracture of the bone or extension of the tumor into the soft parts) tends characteristically to be composed of large aggregates or veritable sheets of more or less compacted cells without any discernible intercellular material and without conspicuous supporting stroma. However, where the bone marrow is being invaded by tumor one observes marrow cells intermingled with tumor cells which, as they proliferate, tend to crowd out and eventually replace the marrow constituents. In selecting material for sectioning, one should choose some blocks of solid tumor tissue (relatively free of secondary changes) which do not require decalcification, since treatment

80. Bichel, J., and Kirketerp, P.: Acta radiol. **19**:487, 1938.
81. Harding, W. G., II, and Kimball, T. S.: Am. J. Cancer **16**:1184, 1932.
82. Rutishauser, E.: Centralbl. f. allg. Path. u. path. Anat. **58**:355, 1933.

with acid tends to shrink the cells and to darken and obscure nuclear detail. Most of our histologic preparations were stained with hematoxylin and eosin, though some were dyed with eosin-methylene blue, which seems particularly well suited to bringing out cytologic detail.

It has been recognized and must be emphasized that the cytologic picture is not the same in all specimens of multiple myeloma. Roughly, however, the tumors can be fitted into two general cytologic groups. On the one hand, there are those in which the tumor cells are quite uniform and predominantly small, and have a superficial resemblance to plasma cells. The tumor cell is roundish and has a stippled nucleus substantially filling the cell. The darkish chromatin particles spotting the nucleus are dispersed centrally as well as peripherally, and one actually observes nothing resembling a cart wheel in the sense of spokes radiating from a hub. The cytoplasm tends to be uniformly eosinophilic, though in occasional tumors one may observe lighter-staining perinuclear demilunes. Interspersed among these cells there may be some cells which, though of the same general character, are larger in respect to both cytoplasm and nucleus. There may also be occasional cells with double nuclei, but there is no tendency to cellular irregularity otherwise. It is to the myeloma showing this cytologic character that the name "plasma cell myeloma" or "plasmacytoma" is commonly applied (although these names have come to be rather indiscriminately applied to most myelomas, even to those in which the tumor cells have only the remotest resemblance to plasma cells) (fig. 9 *A*).

In the other group of myelomas the cytologic picture tends to be dominated by cells larger than those resembling plasma cells, but may be a rather variegated one. The dominant cells in the tumor generally exceed the myeloblast in size and, on the whole, show fairly abundant cytoplasm and have a large, round, oval or even reniform, pale stippled nucleus. The latter is not necessarily eccentric and indeed is often centrally placed. In some tumors the nuclei of certain of the cells may contain a well defined pinkish or reddish round body resembling a nucleolus. The cytoplasm is generally eosinophilic but sometimes takes a more basophilic or polychromatic tinctorial hue, and in some tumors it also presents paler demilunes around the central face of the nucleus. Occasionally the cytoplasm is vacuolated or contains refractile rod-shaped bodies, considered by some to be of protein nature.[83] In any particular tumor site examined, one may also find some of the smaller cells resembling plasm cells or, on the other hand, find cells which are much larger than the dominant ones and frequently show nuclear atypism. Specifically, such atypical cells may present large and hyperchromatic nuclei of bizarre shape, or two or more nuclei (fig. 9 *B* and *C*; fig. 10 *A*, *B* and *C*).

---

83. Steinmann, B.: Deutsches Arch. f. klin. Med. **185**:49, 1939.
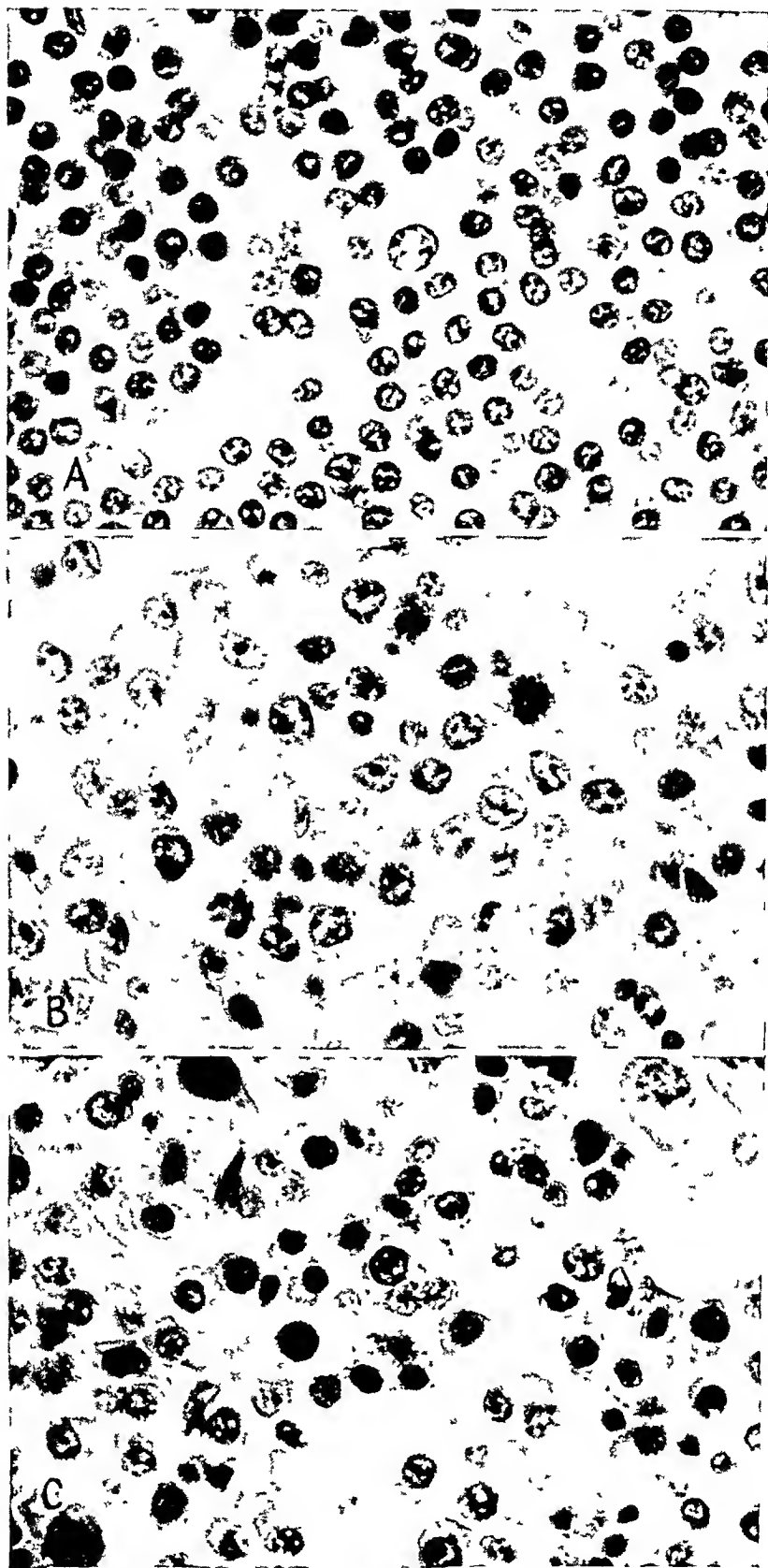
Fig. 9—Photomicrographs illustrating the cytologic character of multiple myeloma. *A,* myeloma composed of predominantly small, uniform tumor cells that have a superficial resemblance to plasma cells. In the center of the field there is a tumor cell with a substantially larger nucleus Myelomas showing this cytologic appearance are commonly designated as plasma cell myeloma or plasmacytoma. × 450.

B, another myeloma whose cytologic appearance is dominated by cells considerably larger than those resembling plasma cells. A number of the large tumor cells show mitotic figures × 450.

C, still another myeloma composed predominantly of larger cells, including some with very large, hyperchromatic nuclei, but showing also an appreciable number of smaller cells, some of which likewise present hyperchromatic nuclei × 450.

Among the tumors in our material there were a number which cytologically seemed to be intermediate between the predominantly small cell and large cell myelomas. These tumors resembled the small cell tumors in that they presented considerable cellular uniformity; however, their cells had somewhat larger nuclei on the whole, and there were an appreciable number of cells with very large nuclei and more than very occasional cells with two or more nuclei.

Correlation of the cytologic appearance of the myelomas in our material with the pertinent biochemical data, especially with the serum protein values (in those cases in which these values had been determined), yielded some interesting results (table). Specifically, we had 4 myelomas classed as large cell myelomas according to the criteria just

Correlation of Cytologic and Pertinent Biochemical Data

	Serum Albumin	Serum Globulin	Bence Jones Proteinuria
Large cell myelomas			
M. H.	2.8	7.6	Negative
A. R.	2.8	3.4	Positive
J. B.	2.0	14.0	Negative
M. K.	3.7	3.9	Negative
F. A.	...	...	Positive
G. F.	...	...	Positive
J. M.	...	...	Positive
M. S.	...	...	Negative
Intermediate group			
M. M.	3.3	6.9	Negative
P. L.	2.7	10.1	Positive
I. K.	...	...	Positive
M. K.	...	...	Negative
N. K.	...	...	Negative
Small cell myelomas			
E. O.	4.2	2.4	Negative
C. J.	4.8	1.9	Negative
N. G.	4.7	1.7	Negative
A. C.	4.1	2.5	Negative
S. B.	4.7	2.5	Positive

outlined and 2 that were intermediate in type, and with all of these the serum globulin values were significantly elevated, while the corresponding serum albumin values were diminished. On the other hand, there were 5 myelomas classed as small cell myelomas by the same criteria, and with all of these the serum albumin and globulin values were well within the normal range. As previously noted, Bence Jones proteinuria was observed with both cytologic types, though it was found more often with large cell myelomas. Hypercalcemia was observed about as frequently with one type as with the other.

It would appear from these data that the cases of large cell myeloma in particular are characterized by hyperglobulinemia. If this trend should be substantiated by further observations, we would have at least some insight into the puzzling question as to why hyperglobulinemia is observed in certain cases of multiple myeloma but not in others. Specifically, it would seem plausible that globulins may be produced or
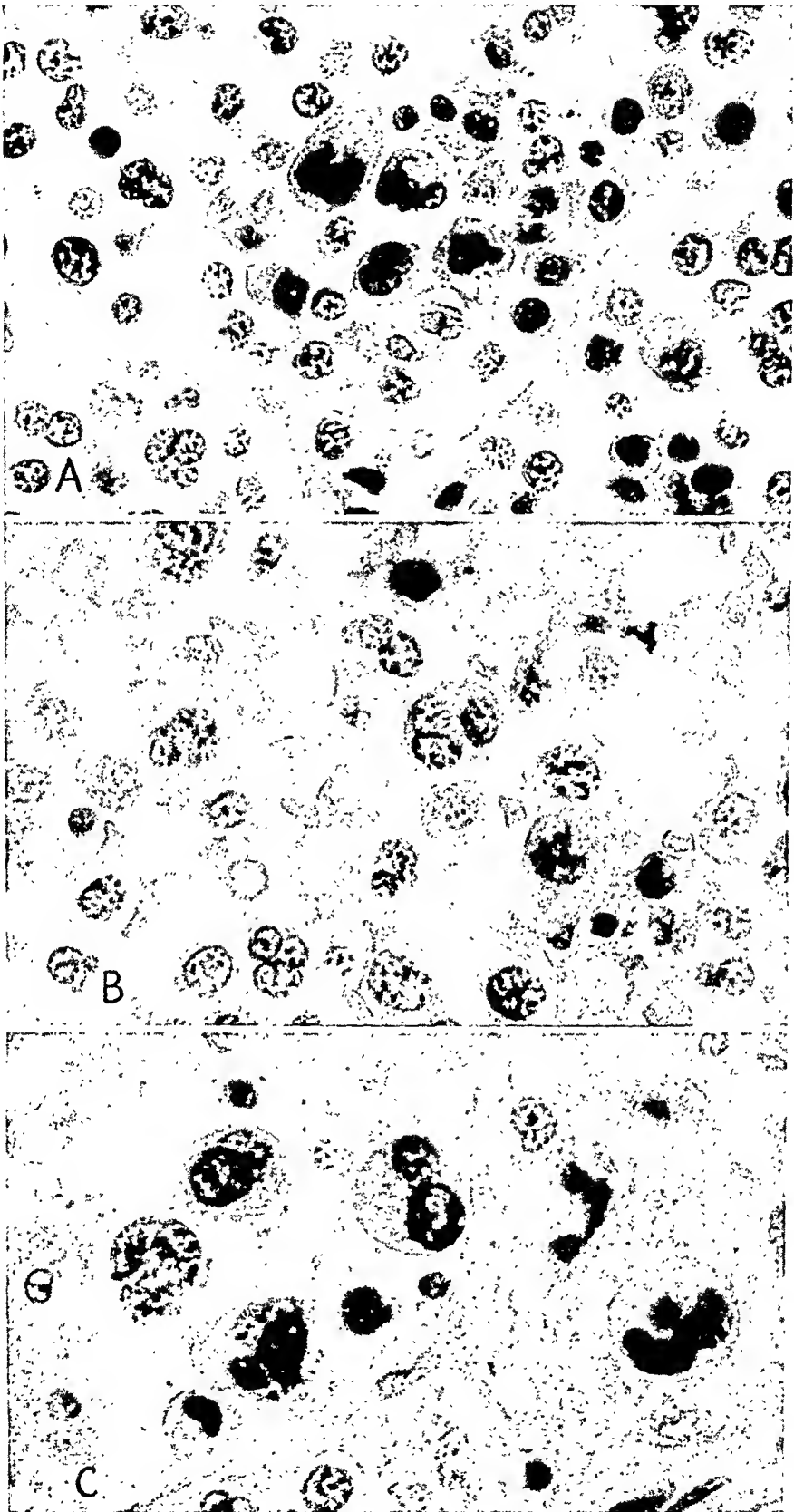
Fig. 10.—Photomicrographs illustrating the cytologic character of multiple myeloma (continued from fig. 9). *A*, a myeloma whose cytologic picture is dominated by comparatively large cells, though there is a sprinkling of smaller cells with nuclei not unlike those of the so-called plasma cells. A number of the cells have two or more nuclei, and some of these multinuclear tumor cells have markedly hyperchromatic nuclei. × 450.

B, another field of the same tumor, showing many cells with multiple nuclei. × 450.

C, another field of the tumor illustrated in *A* and *B*, showing a number of myeloma cells with bizarre hyperchromatic giant nuclei. × 450.

stored in appreciable quantity within the large and apparently less mature tumor cells and, by the same token, liberated and mobilized by way of the blood stream as these cells are broken down.

It is pertinent at this point to consider whether or not the large and small tumor cells respectively represent essentially different types of cells so far as their derivation is concerned. This question may not be of as great practical moment as it would be if, for instance, it had been established that the myelomas of relatively mature histologic appearance respond more favorably to treatment or necessarily pursue a more prolonged course than do the others; it is, however, germane to a better understanding of the nature of the neoplastic process. We are inclined to doubt whether there is any essential difference, save one of maturity, between the large and small tumor cells, since the predominantly large cell myelomas contain smaller cells in some places, to which it is possible to trace transitions, and the predominantly small cell myelomas contain occasional large cells (figs. 9 and 10). We would emphasize instead the unity of multiple myelomas and explain cytologic variations within them as expressions of their relative maturity or immaturity. This is the concept that has been advocated by Wallgren [84] in particular. One may draw a parallel with cancerous lymphomas, among which one observes some composed of relatively small, mature lymphocytic cells, others composed of somewhat larger, less mature lymphoblastic cells and still others composed of quite large, immature reticulum cells of more variable appearance, all of them derived from a common lymphoid stem cell (Mallory).

The identification of the common ancestral cell of multiple myeloma, however, is still a moot point. Some [85] have held that the tumor cells of multiple myeloma are abnormal hematic cells whose origin may be traced to the primitive reticulum cell of the bone marrow, and this theory has much to recommend it. Others have been impressed in certain cases by the alleged resemblance of the tumor cells to myeloblasts or myelocytes,[86] to lymphoblasts or lymphocytes, to erythroblasts,[87] to mature or immature marrow plasma cells,[88] to megakaryoblasts [89] or to hemocytoblasts.[90]

84. Wallgren, A.: Untersuchungen über die Myelomkrankheit, Upsala, Almquist & Wiksell, 1920; Baltimore, William Wood & Company, 1920.

85. Klemperer, P.: Beitr. z. path. Anat. u. z. allg. Path. **67**:492, 1920. Zadek, I.: Folia haemat. **58**:196, 1937.

86. Mieremet, C. W. G.: Virchows Arch. f. path. Anat. **219**:1, 1915. Symmers, D.: Ann. Surg. **67**:687, 1918.

87. Harbitz, F.: Norsk. mag. f. lægevidensk. **84**:212, 1923. Froboese.[25b]

88. Christian, H. A.: J. Exper. Med. **9**:325, 1907. Kracke, R. R.: Diseases of the Blood, ed. 2, Philadelphia, J. B. Lippincott Company, 1941, pp. 82 and 520.

89. Gunn, F. D., and Mahle, A. E.: Arch. Path. **26**:377, 1938.

90. Smith, R. P., and Silverberg, M.: Arch. Path. **21**:578, 1936.

Be that as it may, it should be emphasized that multiple myeloma presents a characteristic clinicoanatomic picture, centered around the skeletal manifestations of the disease and their sequelae, and that, with rare exceptions, this picture is readily distinguishable from that presented by any of the other neoplasms of hemopoietic derivation. So distinctive is multiple myeloma as a single and basically uniform disease complex that one is at a loss to understand why in some quarters it has been subclassified, presumably on the basis of cell type, into plasma cell, myeloid, erythroid and lymphoid myeloma. Indeed, there can be little doubt that multiple myeloma as discussed in this paper is a disease of unitary cell type, the variations in cytologic appearance reflecting stages in the maturation of the basic tumor cell. Specifically, regarding the histogenesis of multiple myeloma, we are inclined to hold with Wallgren,[84] Wood and Lucké,[91] Wintrobe [8] and others that this neoplasm consists of distinctive tumor cells which are probably of myeloid formative or hematic origin (though not clearly resembling any normal marrow cells or their immediate precursors) and are best designated noncommitally as myeloma cells.

Cognizance must also be taken of the idea stemming from Rustizky [92] and Lubarsch [93] that multiple myeloma represents a systematized disease of the hemopoietic apparatus and, as such, is not a true neoplasm but rather a hyperplasia related to the "leukemias." There can be no serious objection to holding that multiple myeloma is akin to chronic myelosis and cancerous lymphoma in the sense that it, too, belongs to the general family of neoplasms of hematic origin, although, as has already been indicated, it presents definite clinicoanatomic characteristics that sharply delimit it from these diseases. However, it is difficult to understand, even on theoretic grounds, how multiple myeloma can be regarded as anything but a cancerous neoplasm in the face of widespread formation of tumors within the skeleton, the tendency toward perforation of the cortices of affected bones and extension into the adjacent soft parts, the capacity of its cells to invade the blood stream and to metastasize to the viscera generally, in addition to involving the hemopoietic organs, and the consistent trend toward a fatal termination.

TREATMENT

Problems in therapy are concerned mainly with palliation, particularly the relief of distressing bone pain, general supportive measures and the handling of such complications as fractures and compression of the spinal cord. In regard to general supportive measures, the use of repeated transfusions to combat anemia when this is present and the avoidance

91. Wood, A. C., and Lucké, B.: Ann. Surg. **78**:14, 1923.
92. von Rustizky, J.: Deutsche Ztschr. f. Chir. **3**:162, 1873.
93. Lubarsch, O.: Virchows Arch. f. path. Anat. **184**:213, 1906.

of prolonged bed care should be emphasized. The consensus of radio-therapists seems to be that roentgen therapy, if judiciously employed, frequently, though not invariably, has value in palliation but that it has relatively little influence otherwise on the course of the disease except possibly when one is dealing with what appears to be a solitary myeloma.

Experience with radioactive phosphorus (P[32]) in the treatment of multiple myeloma is still limited but is sufficient to indicate what may be expected at best and what its limitations are. In some patients, but by no means all, radiophosphorus therapy has resulted in appreciable subjective clinical improvement, evidenced chiefly by relief of pain permitting restoration of more normal activity.[94] Apparently, no con-comitant significant change in the roentgenographic appearance of the skeletal lesions has been observed. Reinhard and associates [95] concluded from their survey that radioactive phosphorus has not proved to be a really valuable therapeutic agent for the treatment of multiple myeloma and that the latter does not respond as favorably to that agent as it does to roentgen radiation. Indeed, it was felt that radiophosphorus therapy had shortened the life expectancy of 2 patients by producing severe leukopenia and thrombocytopenia.

The administration of "stilbamidine" (4, 4-diamidinostilbene) and "pentamidine" (4, 4'-[pentamethylenedioxy] dibenzamidine) in conjunc-tion with a diet low in animal protein has recently been advocated by Snapper[96] for the treatment of patients with multiple myeloma, especially those with widespread but not large osteolytic lesions and with normally functioning kidneys. In such patients (15 had been so treated) he claimed to have observed a favorable influence on excruciating bone pain, but stated that the lesions persist and that treatment at best only checks the disease temporarily and does not cure it. Snapper pointed to the appearance of granules within the cytoplasm of the myeloma cells as an indication of the specific action of "stilbamidine" on these cells. The drug, however, has certain toxic effects, including injury of the trigeminal nerve in some cases, which is manifested in the development, following a delay, of facial anesthesia, and it seems that before employing the drugs in question for their palliative effect, one would be well advised to try first roentgen therapy in order to achieve the same result.

Aside from treatment of fractures, especially those of long bones, surgical intervention has a place in the relief of transverse myelitis resulting from extradural compression, which is accomplished by lami-

94. Low-Beer, B. V. A.; Lawrence, J. H., and Stone, R. S.: Radiology **39**: 573, 1942. Warren, S.: Am. J. M. Sc. **209**:701, 1945.

95. Reinhard, E. H.; Moore, C. V.; Bierbaum, O. S., and Moore, S.: J. Lab. & Clin. Med. **31**:107, 1946.

96. Snapper, I.: J. A. M. A. **133**:157, 1947.

nectomy. In regard to the latter, Jacox and Kahn [97] and Batts [4] have shown that this procedure, if followed by roentgen therapy, may permit complete recovery of function and even survival thereafter for a number of years. They emphasized that laminectomy should always be done before roentgen therapy is given, in order to prevent further damage being done to the cord by swelling subsequent to irradiation. Also, the question of ablation of a limb for myeloma sometimes arises, but only in connection with the comparatively rare, ostensibly solitary myeloma of a long bone. In cases of this type, as noted, one can never be certain that tumor is not present in other bones in spite of their negative roentgenographic appearance. It is pertinent, however, to cite the remarkable case reported by Stewart and Taylor [6c] : The patient was alive and well eight years after forequarter amputation for a huge myeloma which had largely destroyed the upper third of the shaft of a humerus and was freely invading the muscles of the upper arm.

SUMMARY AND CONCLUSIONS

This study (based on 35 proved cases, in 18 of which autopsies were made) places emphasis on the clinical and anatomic features that characterize multiple myeloma. We conceive of the latter as a distinctive malignant disease of the skeleton primarily, which apparently takes its departure from the myeloid formative tissue proper. Anatomically, practically every bone may ultimately come to be involved more or less in a given case. The skeletal progress of the disease may be steady and rapid, sometimes from the beginning and sometimes after a static period. In some cases, also, before the disease becomes spread over the skeleton it may flourish in one bone (as a so-called solitary myeloma) for months or even years. Though at autopsy the skeleton may be found riddled through with foci of myeloma, it is only infrequently that gross foci are found in the viscera and other extraskeletal parts. Nevertheless, even in the absence of gross infiltrations, microscopic examination sometimes reveals smaller or larger numbers of myeloma cells within the spleen, the liver or lymph nodes and occasionally in other organs as well. Also, in some cases, myeloma cells may invade the blood stream. Ordinarily, under these circumstances, relatively few myeloma cells are found in the blood smears, but in an occasional case they may be so numerous as to create a leukemic blood picture (so-called plasma cell leukemia).

Although there are these points of resemblance to other neoplasms of hematic origin, it should be emphasized that multiple myeloma presents a characteristic clinicoanatomic picture, centered around the skeletal manifestations of the disease and their sequelae, and that with rare

---

97. Jacox, H. W., and Kahn, E. A.: Am. J. Roentgenol. **30:**201, 1933.

exceptions this picture is readily distinguishable from that presented by any of the other neoplasms of hemopoietic derivation. In this connection we have stressed the diagnostic significance of hypercalcemia, hyperglobulinemia (and its associated hematologic manifestations) and Bence Jones proteinuria, the not infrequent presence of atypical amyloidosis in association with myeloma and the well known cytologic renal changes of almost pathognomonic distinctiveness. The latter result commonly in more or less heavy albuminuria and often in renal insufficiency of a peculiar type. In regard to amyloidosis it was indicated that multiple myeloma is so often the basis for atypical amyloid deposits that the possibility of myeloma should be investigated in every case of idiopathic amyloidosis, even though the bones present no evidence of tumor either roentgenographically or on gross inspection at autopsy.

The tumor tissue in multiple myeloma tends characteristically to be composed of large aggregates of more or less compacted cells without any discernible intercellular material and without conspicuous supporting stroma. It has been recognized and must be emphasized that the cytologic picture is not the same in all specimens of multiple myeloma. Roughly, however, the tumors can be fitted into two general cytologic groups. On the one hand, there are those in which the tumor cells are quite uniform and predominantly small and have a superficial resemblance to plasma cells. It is to the myeloma showing this cytologic appearance that the name "plasma cell myeloma" or "plasmacytoma" is commonly applied. In the other group of myelomas the cytologic picture tends to be dominated by cells larger than those resembling plasma cells, but may be a rather variegated one. The dominant cell shows fairly abundant cytoplasm and has a large, round, oval or even reniform, pale stippled nucleus. In any particular tumor site examined, one may also find some of the smaller cells resembling plasma cells, or, on the other hand, find cells which are much larger than the dominant cells and frequently show nuclear atypism. Specifically, such atypical cells may present large and hyperchromatic nuclei, giant nuclei of bizarre shape, or two or more nuclei.

We are inclined to doubt whether there is any essential difference, save one of maturity, between the large and the small tumor cells. So distinctive is multiple myeloma as a single and basically uniform disease complex that one is at a loss to understand why in some quarters it has been subclassified, presumably on the basis of cell type, into plasma cell, myeloid, erythroid and lymphoid myeloma. Indeed, there seems to be little doubt that multiple myeloma as discussed in this paper is a disease of unitary cell type, the cytologic variations reflecting stages in the maturation of the basic tumor cell. Specifically, regarding the histogenesis of multiple myeloma, we are inclined to hold with Wallgren and others that this neoplasm consists of distinctive tumor cells which

are probably of myeloid formative or hematic origin (though not clearly resembling any normal marrow cells or their immediate precursors) and are best designated noncommittally as myeloma cells.

Correlation of the cytologic aspects of the myelomas in our material with the pertinent biochemical data strongly suggests that it is the large cell myelomas particularly which are characterized by hyperglobulinemia and, rather often, by Bence Jones proteinuria.

On the clinical side, in our cases of multiple myeloma, we found that the great majority of the patients were between 40 and 60 years of age, though some were in their 30's, and one was only 13 when the first manifestations of the disease appeared. Our data indicate that multiple myeloma may be slightly more prevalent in males than in females but do not support the often repeated statement that the condition is at least twice as frequent in males. In regard to the roentgenographic findings, we found that the picture conventionally held to distinguish multiple myeloma—that of many bones, including the calvarium, riddled by clearcut punched-out osteolytic defects—represents the exception rather than the rule and applies only to certain cases in which the disease is far advanced. Indeed, very often one observes merely some vaguely defined rarefactions in a number of the bones or a single exuberant tumor focus in some one bone (commonly a femur or a humerus, but sometimes a vertebral body, a rib or a clavicle, an innominate bone, a bone of the calvarium or some other bone) without obvious involvement of the skeleton generally. Sometimes (when myelomatous infiltration of the marrow is diffuse) skeletal changes may not be apparent at all roentgenographically, or the replacement of the marrow by tumor may be reflected merely by some osteoporosis. As for the calvarium, this not infrequently fails to show numerous punched-out rarefactions, even when roentgenograms show clearcut and widespread involvement of the rest of the skeleton. In such equivocal or initially obscure cases one must utilize fully all the available diagnostic cues to arrive at a combination of significant findings constituting probable or conclusive evidence of the presence of multiple myeloma. Marrow obtained by sternal puncture is often of great value in establishing the diagnosis.

Multiple myeloma has too variable a clinical course to permit of any dogmatic statement in regard to prognosis. It is true that the average length of survival after the onset of symptoms is not likely to be more than about two years. However, there are occasional patients with multiple myeloma, particularly those in whom the disease was apparently localized at the outset, whose course may be protracted over a number of years, sometimes as long as ten years or more. Problems in therapy are concerned mainly with palliation, particularly the relief of distressing bone pain, and general supportive measures, also the handling of such complications as fractures of bones and compression of the spinal cord.

MECHANISM OF LIPOPHAGE DEPOSITION IN ATHEROSCLEROSIS

ISRAEL GORDON, M.D., M.R.C.P., D.P.H.

ILFORD, ESSEX, ENGLAND

THERE are probably few diseases on which there have been more experimentation and speculation than atherosclerosis, and the result has been the accumulation of a vast and sometimes incoherent collection of facts, for which in many cases exactly opposed explanations have been given. For some years now I have been of the opinion that a simple and rational explanation is available for the main features of the disease and that the published work of Leary provides the last link in a chain of evidence that began with Thoma fifty or more years ago. In brief, Leary [1] suggested that when, through feeding, hypercholesteremia develops in rabbits the cells of the reticuloendothelial system take up the esterified cholesterol and after a latent period become detached, enter the blood stream, pass through the lungs to the systemic circulation and then, in the aorta, pass into the intima. That the reticuloendothelial system when stimulated by "colloid dyes and suspensoids" will deliver macrophages into the blood stream after a latent period was shown some time ago by Evans.[2] Leary [1] in his earlier papers suggested that entrance of the aortic intima was due to chemotaxis and that the site was chosen because of various stresses, but more recently [3] he has suggested that there is a certain sluggishness of the blood current in the aorta and the coronary arteries which causes the sticky macrophages to adhere to the intimal endothelium. Leary has, however, failed to pay full attention to a phenomenon that has been known for about a century and is a commonplace in all textbooks of pathology since Donders [4] and Gunning [5] (quoted by Thoma [6]) pointed it out, i. e., the fact that the corpuscular elements of the blood are in

From the Public Health Department.

1. Leary, T.: Arch. Path. **32**:507, 1941.

2. Evans, H. M.: Am. J. Physiol. **37**:243, 1915.

3. Leary, T.: Am. J. Path. **22**:634, 1946.

4. Donders, F. C.: Physiologie des Menschen, Leipzig, S. Hirzel, 1856, vol. 1; Nederl. Lancet, 1856, vol. 5; cited by Thoma.[6]

5. Gunning: Arch. f. d. holländ. Beitr. z. Nat.-u. Heilk, 1857, vol. 1; cited by Thoma.[6]

6. Thoma, R.: Text Book of General Pathology and Pathological Anatomy, London, Adam & Charles Black, 1896.

the central or axial stream, while the peripheral stream or *Rand-zone* is clear of cells, that, furthermore, the faster the blood stream is the wider is the clear *Randzone* (Sandison [7]) and that, as is well known, the mean velocity of the blood stream is higher in the aorta (300 mm. per second, according to Cowdry [8]) than in any other vessel. It also appears that Leary has no explanation to offer, except the vague term "chemotaxis," as to why the lipophage, and not the other white cells of the blood, forms the main feature of atheroma.

<div align="center">DIAPEDESIS</div>

The problem to be faced is: Which influences bring the lipophages into the clear zone in contact with the endothelium, or, what amounts to the same thing, do away with the clear zone? First, it may be stated that studies on diapedesis since Schklarewsky [9] (quoted by Thoma [6]) and the more recent work of Clark and Clark [10] and Sandison [7] have shown that the most important factor in bringing the corpuscles into the peripheral zone is slowing of the blood stream and that the lighter the corpuscle the earlier it moves out. Schklarewsky, working under the direction of Recklinghausen and Helmholtz, demonstrated in glass tubes that in all fluids which hold in suspension small particles of unequal specific gravity, the heavier particles are collected near the axis of the stream, while the lighter particles circulate near the wall of the vessel. Furthermore, Schklarewsky proved that if the blood flowed in glass tubes which had contractions and dilatations in the course of their lumens, numerous white corpuscles accumulated in the dilated parts, in which the current was slower. It is further suggested that any small part of the arterial wall that projects into the axial stream will cause the peripheral stream at that site to cease to exist. A third factor that would destroy the differentiation between axial and peripheral zones would be any marked eddying.

Although mean blood velocity is at its highest in the aorta, a further analysis of the tendencies of the current in that vessel and in the large elastic arteries shows that the problem is not so simple. As Leary [3] pointed out, cardiac systole and diastole produce a certain intermittency of the stream. The aorta and the large elastic arteries act as a distensible reservoir that fills during systole and partially empties during diastole. This aortic reservoir is itself larger than the capacity

7. Sandison, J. C.: Anat. Rec. **54**:105, 1932.

8. Cowdry, E. V., in Arteriosclerosis, New York, The Macmillan Company, 1933.

9. Schklarewsky: Arch. f. d. ges. Physiol. **1**:602 and 657, 1868; cited by Thoma.[6]

10. Clark, E. R., and Clark, E. L.: Am. J. Anat. **57**:385, 1935.

of the left ventricle (Sainsbury [11]) ; so all its contents cannot leave it in each cardiac cycle—a portion must remain. In fact, some of the blood near the aortic cusps must reverse its direction when the valves are shut during diastole, and blood cannot escape out of the aorta into the common iliac arteries at a maximum rate, for then there would not be sufficient to insure an adequate supply to the more important common carotid arteries. Peripheral resistance in the vessels of the trunk and the limbs must be such as to insure retention of pressure in the aorta sufficient to provide enough blood for the brain despite the assumption of the upright posture by man. In other words, every particle of blood does not move in the aorta at the mean velocity characteristic of that vessel. This intermittency of some portions of the blood in the current gives an opportunity for the lighter corpuscular elements to enter the peripheral zone during diastole, while the heavier elements, owing to greater kinetic energy, have not the same tendency. These factors also apply to a lesser extent to the other elastic arteries. Widening of the vessel, the result of age or of disease, will further lessen the velocity and allow corpuscles to enter the marginal zone that otherwise would not have done so. The selection of the sites of atheroma will be discussed in further detail later.

The lipophage having entered the peripheral zone, it is necessary to suggest an explanation why it, and not the polymorphonuclear leukocyte, enters the lesion of atheroma, for the polymorphonuclear cells vastly outnumber the lipophages. Perhaps an indication is given by a consideration of which property of a corpuscle enables it to leave the axial current before another cell. That property is lightness. The lipophage, as it is packed full with fatty esters of a low specific gravity, must undoubtedly be lighter than the other leukocytes and so would tend to enter the peripheral zone at an earlier stage. Another factor to be considered would be its relative inertia as compared with the polymorphonuclear leukocyte. Leary [1] declared it to be ameboid, as it undoubtedly is, but it would be unnecessarily speculative to assume that this obese cell, stuffed so full of lipids that it resembles a honeycomb with barely distinguishable intervening strands of protop'asm, has the spontaneous activity of a polymorphonuclear leukocyte or other macrophage ; it is probably far more subject to external influences, one of which would be the blood pressure. Should this cell, perhaps adherent to the endothelium, then pass a tentative pseudopod—why, one cannot speculate—into the intima, it would be subject to a difference of pressure on its surfaces, higher inside the blood stream, lower in the intima. As it is of fluid composition and relatively inert, its substance will tend to

---

11. Sainsbury, H.: The Heart as a Power Chamber, London, Frowde, Hodder & Stoughton, 1922.

flow from the higher pressure to the lower, i e., into the intima. It would of course be naive to assume that the pressure in the intima is zero while that in the blood stream is, say, 100 mm. of mercury; nevertheless it can be assumed that in the intima there is appreciably less pressure than in the lumen of the aorta. Proof of this is the demonstration by Winternitz and associates [12] that there are extensive capillary vasa vasorum in the aortic intima, originating from the lumen, particularly that of the older and diseased vessel. The pressure in these vasa must be less than that in the aortic lumen or blood would not flow into them, and the hydrostatic pressure in these vessels must be greater than that in the surrounding tissue or they would not function; therefore, a fortiori, the pressure in the intimal tissues must be less than that in the aorta. Winternitz and associates also demonstrated extensive hemorrhages originating from these vasa, in the intima, thus giving additional proof that the intimal pressure is less than the aortic. A similar conclusion must result from consideration of the dissecting aneurysm. However, the difference in pressure cannot be excessive, for then the aortic pressure would cause the intimal vasa to collapse. Blood pressure can thus be said to be a factor in the cause of atheroma, but not necessarily high blood pressure, merely the normal arterial pressure.

FATE OF THE LIPOPHAGE IN THE INTIMA

The lipophage having entered the intima, one finds the earliest sign of atheroma. As described by Leary, this is not even macroscopic, and one of the most contentious aspects of the disease has been the difficulty of determining which picture comes first, but Leary has extensive support, from as far back as Klotz and Manning [13] to as recent as Sjövall and Wihman,[14] whose material consisted of 1,380 cases, that the lipid is first of all intracellular. The lipophage, having entered the intima, would tend to move peripherally, no doubt helped by the same pressure influences, until it arrived at the internal elastic lamella. This structure, described by Dees [15] as a "feltwork" or "an exceedingly close-meshed plexus of very fine elastic fibres giving the appearance of a solid film-like sheet," has been described already by Aschoff [16] and Rosenthal [17] as a barrier to medial deposition of lipids of colloid form. How much more

12. Winternitz, M. C.; Thomas, R. M., and le Compte, P. M.: The Biology of Arteriosclerosis, Springfield, Ill., Charles C Thomas, Publisher, 1938.

13. Klotz, O., and Manning, M. F.: J. Path. & Bact. **16**:211, 1911.

14. Sjövall, H., and Wihman, G.: Acta path. et microbiol. Scandinav., 1934, supp. 20, p. 1.

15. Dees, M. B.: Anat. Rec. **26**:161, 1923.

16. Aschoff, L.: Brit. M. J. **2**:1131, 1932.

17. Rosenthal, S. R.: Arch. Path. **18**:473 and 668, 1934.

would it be a barrier to the obese lipophage, whose natural egress from any situation is the lymphatic channel! In the aorta there are in fact 50 to 65 of these concentric barriers (Maximow and Bloom [18]). It is not suggested that the internal elastic lamella is an absolute obstacle, for it is fenestrated, and some cells make their way into the media, especially in those places where the elastica is less complete (Aschoff [16]), and can be found in the lymphatic channels of the adventitia (Leary [19]). Fox [20] stated that in the bird the internal elastic lamella is deficient and the lesion tends to extend more into the media. For the majority of these cells, however, imprisoned by the blood pressure on one side and the internal elastic lamella on the other, the intima is a mausoleum, and the further changes that occur therein need not be described in this paper.

WHY THE DISEASE AFFECTS MAINLY THE ELASTIC ARTERIES

If it is true that slowness of the blood is one of the main factors in the deposition of the lipophages, it must be clearly explained why the disease does not occur in other parts of the vascular system. First: Although the mean velocity of the blood gets less as the arteries become smaller, beyond the elastic arteries and especially in the veins the blood travels in a more even manner, less intermittently, than in the aorta and larger vessels; so the distinction of axial and peripheral currents tends to remain. In the veins the velocity, of course, increases. Second: Even if a lipophage should wander into the peripheral stream, it is required that the pressure be sufficiently high to push it out of the lumen. There is a fall of blood pressure from the aorta onward; the pressure, in fact becomes negative in the largest veins. Clark and Clark [10] described how a macrophage can even move from the tissues into the lumen of a capillary under the conditions of pressure existing therein. Thus the lower the pressure in a vessel the less the tendency for atheroma to occur. Third: Once the lipophage enters the intima it must be imprisoned therein by an internal elastic membrane. There are over fifty of these in the aorta, one in a muscular artery, and in an arteriole and a capillary none. In these small vessels the lipophage on leaving the lumen can easily find its way to a lymphatic channel except in tissues where there are none. If too many leave a capillary at any spot and the lymphatic vessels cannot cope with this, a xanthoma is the result. Such lesions have been caused in hypercholesteremic rabbits by trauma (Anitschkow [21]). If there are no lymphatic vessels, the

18. Maximow, A. A., and Bloom, W.: Text Book of Histology, Philadelphia, W. B. Saunders Company, 1943.

19. Leary, T.: Arch. Path. **21**:419, 1936.

20. Fox, H., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933.

21. Anitschkow, N.: München. med. Wchnschr. **60**:2555, 1913.

lipophages die eventually, leaving the cholesterol; this occurs in the cornea in arcus senilis, a feature not only of the elderly person but also of cholesterol-fed rabbits. In the veins the internal elastic lamella is far less well developed than in the arteries, and the vessel wall is also more vascular. These three features, i. e., more continuous flow, lower pressure and absence or incompleteness of elastic membranes, suffice to explain why atheroma is mainly a disease of the elastic arteries.

An attempt will now be made to show how the main pathologic observations and experimental results relating to atherosclerosis fit in with the hypothesis that has been developed.

LOSS OF ELASTICITY, DILATION AND LESSENED VELOCITY

Of all the facts relating to atherosclerosis the one on which there is most agreement is that with age the aorta tends to dilate as a result of loss of elasticity. Among the many authors who agree on this are: Adami,[22] Blumenthal and co-workers,[23] Duff,[24] Hueper,[25] Krafka,[26] Ophüls,[27] Plesch,[28] Rosenthal,[17] Thoma,[6] Wells,[29] Wilens,[30] Winternitz and associates.[12] This medial weakness is ignored only by the most enthusiastic cholesterol advocates and is the main argument in the armory of the anticholesterol protagonists, such as Duff.[24] Blumenthal and associates [23] have shown that it is accompanied by increased deposition of calcium in the media, as disclosed by microincineration, and Wilens [30] has demonstrated in postmortem material that this loss of elasticity is accompanied, where it occurs, by atheroma. If a vessel dilates, other things being equal, the velocity diminishes, for

$$V = \frac{A}{\pi\, r^2}$$

where V equals velocity in seconds and A equals volume per second. Using this formula and circumferences of aortas given by Kaufman and Aschoff, quoted by Krafka,[26] one finds that the average velocity of the blood of the aorta at 50 years of age is 60 per cent of that at 20 years of age. (This relation is illustrative only; it may not exactly

22. Adami, J. G.: Am. J. M. Sc. **138**:485, 1909.

23. Blumenthal, H. J.; Lansing, A. I., and Wheeler, P. A.: Am. J. Path. **20**:665, 1944.

24. Duff, G. L.: Arch. Path. **20**:81, 1935.

25. Hueper, W. C.: Arch. Path. **38**:162, 1944.

26. Krafka, J.: Arch. Path. **23**:1, 1937.

27. Ophüls, W., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933.

28. Plesch, J.: Lancet **1**:385, 1932.

29. Wells, H. G., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933.

30. Wilens, S. L.: Am. J. Path. **13**:811, 1937.

parallel the conditions in vivo.) Many years ago Thoma[6] suggested that a sluggish blood flow caused arteriosclerosis, without stating just how it happened, and he has been constantly criticized ever since. Adami,[22] for instance, stated that he had "never been able to grasp his explanation; furthermore I have never met anyone who has pretended to do so." Hueper[25] dismissed his suggestion that the lesions occur in situations where the velocity is lessened by pointing out that atherosclerosis is a disease of the aorta and not of vessels where the velocity is diminished. This point has, effectively, it is hoped, been dealt with earlier. There is no doubt that Thoma did not know why sluggish blood flow was so important, for the work on cholesterol feeding of rabbits had not been started and the lipophage theory had not been enunciated. Nevertheless, he took a great part in the description of the various phenomena connected with the leukocytes migrating from vessels and described Schklarewsky's work in which white corpuscles collected in the wider parts of glass tubes whose width varied. Thoma, furthermore, insisted that the particles of blood in actual contact with the wall of the vessel did not move at all. Not only is the general dilatation important, but local dilatations, i. e., aneurysms, are the site of atheroma. In experimental animals as well as in human subjects this is so—as, for ·instance, in the aneurysms of chicks described by Dauber and Katz,[31] in which atheroma occurred; in the fusiform dilations of the aorta described by Liebig,[32] and in the femoral artery of the side subjected to sympathectomy in the rabbit described by Harrison.[33] It should be remembered that atheroma is not usually confined to these places, but the incidence is greater there. The fact that in the adult the atherosclerosis is more marked in the abdominal aorta is to be correlated with the loss of elasticity (Wilens[30]) and the consequent greater relative widening there, this loss of elasticity perhaps being due to the extra head of pressure added to the normal pressure of the blood of the aorta on the assumption of the vertical posture. Be it noted that in the human infant the atheromatous streaks are mainly in the arch and the root of the aorta, and the older the subject the more the lesion spreads to the abdominal aorta (Ashoff[16]). Thus in the nonupright human being the site of atheroma is much the same as that in the rabbit, an effective answer to one of the main criticisms, that the rabbit lesion is not comparable with the human. Thus the theory that medial weakness and disease cause atheroma does not oppose the cholesterol theory; they are complementary to each other.

31. Dauber, D. V., and Katz, L. N.: Arch. Path. **36**:473, 1943.
32. Liebig, H.: Arch. f. exper. Path. u. Pharmakol. **159**:359, 1931.
33. Harrison, C. V.: J. Path. & Bact. **48**:253, 1939.

ATHEROMA AT THE SITE OF BRANCHING

In the rabbit and in man the lesion is well known to occur at the sites where arteries leave the aorta.   In an artery that branches off at a right angle the atherosclerosis tends to surround the mouth of the vessel.   When any particle changes its direction suddenly its momentum must slacken, and furthermore, eddies must occur here, both circumstances tending to bring the lipophage in contact with the endothelium. The lesion differs somewhat when the branch goes off at an oblique angle, for here the spur between the two vessels is the most affected. This would be expected, for when a branch goes off obliquely the axial and peripheral currents tend to remain separate, the lipophage remains in the axial stream, but the spur between the two vessels itself is in the axial stream.   The striking and distortion of leukocytes against a spur of a dividing vessel are well described as observed in the living animal by Clark and Clark.[10]

OTHER ASPECTS OF BLOOD VELOCITY

As age advances, it does not appear that blood velocity lessens (Blumgart and Weiss [34]), although the incidence of atheroma increases. However, it is not the velocity of the blood of all the systemic and pulmonary vessels in which we are interested, but merely the velocity of that of the elastic arteries, which by virtue of their dilation, have a slower blood flow in old age.   Presumably, the compensation that allows for a normal circulation time lies in the smaller vessels.   High blood pressure is often associated with atherosclerosis, although it has been pointed out repeatedly that the disease regularly occurs with normal pressure.   The blood velocity in fully compensated cases of high blood pressure is never increased; it is either normal or retarded (Blumgart and Weiss [35]).   This is interesting, as the velocity of fluid in a tube, according to Poiseuille's Law, increases with increased difference of pressure between the ends of the tube.   Thus in the body there must be compensations; probably an increased speed of flow in the smaller vessels plus the slower speed in the elastic arteries gives an average or slightly lengthened circulation time.

There is one condition which is constantly associated with changes in circulation time in man, and that is the basal metabolic rate.   With an increased basal metabolic rate, i. e., in hyperthyroidism, the circulation time is diminished, and in hypothyroidism it is increased (Stewart and Evans [36]; Blumgart [37]; Tarr and co-workers [38]).   This

34. Blumgart, H. L., and Weiss, S.:   J. Clin. Investigation **4**:15, 1927.
35. Blumgart, H. L., and Weiss, S.:   J. Clin. Investigation **4**:173, 1927.
36. Stewart, H. J., and Evans, W. F.:   Am. Heart J. **23**:175, 1942.
37. Blumgart, H. L.:   Medicine **10**:1, 1931.
38. Tarr, L.; Oppenheimer, B. S., and Sager, R. V.:   Am. Heart J. **8**:766, 1933.

brings one immediately to the experimental work on cholesterol feeding of rabbits combined with manipulations of the thyroid apparatus, commenced by Murata and Kataoka [39] and followed up by others, such as Turner,[40] the gist of which is that the administration of thyroid diminishes experimental atherosclerosis and thyroidectomy increases it. The former would speed up the circulation, and the latter would diminish it. Unfortunately, the velocity of the blood is not the only factor involved in these experiments, for the blood cholesterol is changed as well, so the changes of circulation time are only part of the story. Turner and Khayat [41] have shown that administration of potassium iodide or of whole thyroid protects the experimental rabbit but that potassium iodide does not do so in the absence of the thyroid gland; thus potassium iodide does so by stimulating the gland. This leads up to the work of Page [42] on the di-iodide of ricinstearolic acid, a substance which prevents atheroma but increases blood cholesterol. If it can be shown that this substance stimulates the thyroid gland (the animals lost weight), it will be a pretty demonstration of the influence of blood velocity on atheroma with diminution of blood cholesterol excluded. However, the effect of iodine compounds on the thyroid gland is complex (Rosenthal [43]), and little can be assumed. The latest development, reported by Steiner and Kendall,[44] is of great interest, for by lowering the basal metabolic rate in dogs with thiouracil they could cause atherosclerosis after cholesterol feeding with regularity. This is one more nail in the coffin of the statement that experimental cholesterol-feeding arterial disease has no relation to human atheroma as it can be produced only in rabbits. From the human angle this demonstrates a danger of inducing coronary thrombosis in patients with thiouracil, one such case at least having already been reported in the literature (Himsworth and associates [45]). Another substance that increases the blood velocity is epinephrine, by action on the heart itself (Blumgart [37]). Anitschkow [46] described experiments in which cholesterol feeding of rabbits was combined with administration of epinephrine. The result was to prevent deposition of atheroma at the normal sites but to produce it at the sites of medial damage characteristic of the action of epinephrine.

39. Murata, M., and Kataoka, S.: Tr. Jap. path. soc. 8:221, 1918.

40. Turner, K. B.: J. Exper. Med. 58:115, 1933.

41. Turner, K. B., and Khayat, G. B.: J. Exper. Med. 58:127, 1933.

42. Page, I. H.: Biol. Symposia 11:43, 1945.

43. Rosenthal, S. R.: Arch. Path. 18:827, 1934.

44. Steiner, A., and Kendall, F. E.: Arch. Path. 42:433, 1946.

45. Himsworth, H. P.; Morgans, M. E., and Trotter, W. R.: Lancet 1:241, 1947.

46. Anitschkow, N., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933.

VASA VASORUM OF THE INTIMA

Winternitz and co-workers [12] ascribed atherosclerosis to hemorrhages and their sequelae, originating from extensive capillary networks in the intima. Vascularization of the diseased intima was described before by Robertson [47] and Paterson,[48] who denied that the vessels were present in the young healthy intima; even Winternitz and associates declared that in the latter circumstances vessels are sparse. Dauber and Katz [31] combined this theory with that of Leary, suggesting that the vasa vasorum become plugged with lipophages and so the intimal lesion is caused. Neither group of workers has considered the aorta of the young rabbit, in which the intima consists merely of an endothelial lining on the internal elastic lamella (Fox [20]). Nobody has described vasa vasorum of this site; if there were any, their presence would appear quite unnecessary; nevertheless, atheroma occurs there. This is not to deny the validity of the observations of Dauber and Katz. What they have described is the phenomenon of "leukocyte skimming" observed in the living animal by Sandison.[7] When the marginal stream is clear of leukocytes, capillaries leading off from the main vessel carry away only plasma, the process known as plasma skimming. Should the velocity in the main vessel have slackened to such an extent that leukocytes have passed into the marginal stream, the blood entering the capillaries carries with it leukocytes and plasma, and Sandison called the phenomenon leukocyte skimming. Dauber and Katz in their paper mentioned stagnation of the blood supply as a reason why the lipophage enters the capillary vasa, but they did not consider this lessened velocity (probably the most fundamental phenomenon in the causation of atherosclerosis) important enough to include it in their conclusion, and in a later review of the subject the same authors did not mention speed of circulation at all (Katz and Dauber [49]). These intimal vessels anastomose with adventitial ones, passing through the media. This gives an adequate explanation of lesions in the media featuring lipophages resulting from medial disease or experimental devices as described by Duff [24] and Ssolowjew.[50] Moon [51] formulated his anemia theory of atheroma, which he stated is the result of collapse of vasa vasorum caused by high aortic pressure. Winternitz and associates,[12] however, held that atheroma is the result of bleeding from extensive capillary networks, a precisely opposed conclusion. That the last-named authors entirely failed to consider the effects of pressure is shown by

47. Robertson, H. F.: Arch. Path. **8**:881, 1929.
48. Paterson, J. C.: Arch. Path. **22**:313, 1936
49. Katz, L. N., and Dauber, D. V.: J. Mt. Sinai Hosp. **12**:382, 1945.
50. Ssolowjew, A.: Ztschr. f. d. ges. exper. Med. **69**:94, 1929.
51. Moon, V. H.: Arch. Path. **3**:404, 1927.

their description of a sinusoid of the intima projecting into the lumen of the aorta like a hemorrhoid, which is of course impossible, as the pressure in an intimal vessel cannot be greater than that in the aorta.

BLOOD PRESSURE AND ATHEROSCLEROSIS

It has been stated by many authors, of whom Moschowitz[52] is one of the latest, that high blood pressure is the main cause of athero-sclerosis, in spite of evidence to the contrary. They have cited the incidence of lesions in the pulmonary vessels in mitral disease, emphysema, etc., in the aorta proximal to the constriction in coarctation of the aorta, and in the aorta of the rabbit on the near side of an experimental narrowing, as well as the incidence of sclerosis of portal and mesenteric veins in hepatic cirrhosis, as proof of the high pressure theory. But the vessel proximal to a constriction always dilates if given time, so the velocity of its blood current is reduced, and this factor is consistently ignored, although it is present where high blood pressure cannot be, as in an aneurysmal dilation due to weakness of the wall of a vessel. The blood pressure in the pulmonary artery at its greatest, in mitral stenosis with heart failure, does not reach that in the normal aorta (Bloomfield and associates[53]); yet that artery can be extensively sclerosed. The pressure in a vessel must be high enough to provide a vis a tergo to push a lipophage into the intima, but need not exceed normal arterial pressures.

STRESS AND STRAIN

A bibliography of writers who have ascribed the localization of atheroma to sites of excessive stress and strain would run into some hundreds. However, the use of these terms is in reality a confession of ignorance and explains nothing. These terms are commonly used elsewhere in discussions of pathologic alterations of elastic tissue, such as hypertrophic emphysema of the lung, for which again a logical mechanical explanation can be given (Gordon[54]).

FIXATION AND MOBILITY

Although the sites of atherosclerosis are well known and noncontroversial, it is strange that two completely opposed views exist, one that the lesion occurs at sites of mobility (Harrison[55]), the other that it occurs at sites of fixation (Wilens[56]). There can be no questioning of

52. Moschowitz, E.: Am. J. M. Sc. **178**:244, 1929.
53. Bloomfield, R. A.; Lanson, H. D.; Cournand, A.; Breed, E. S., and Richards, D. W.: J. Clin. Investigation **25**:639, 1946.
54. Gordon, I.: Dis. of Chest **10**:180, 1944.
55. Harrison, C. V.: J. Path. & Bact. **36**:447, 1933.
56. Wilens, S. L.: Am. J. Path. **18**:63, 1942.

the experimental findings of these authors; it is their interpretations that must be at fault.   Surely the early fatty streaks observed in the aorta are at sites of neither excessive fixation nor mobility.   One might explain Harrison's findings in the terms of this paper by saying that intimal deposition occurred in the noncalcified parts of the aorta in his rabbits because the blood pressure in systole pushed those parts of the aorta out, caused relative widening of the vessel there and the lipophages entered the marginal zone of the slower stream.   Atheroma occurred at the site of the silver cuffs of Wilens because in systole these projected into the axial stream.   A similar explanation suffices for the deposition of atheroma where fractures occurred in the calcified media in Harrison's rabbits.   The matter of eddies must also be considered but is difficult to interpret.

MACROMOLECULAR SUBSTANCES OF HUEPER

The interesting studies of Hueper[57] in which he injected at various times polyvinyl alcohol, pectin, acacia, methyl cellulose, sodium cellulose glycollate and hydroxyethylcellulose into experimental animals and caused atheromatous lesions in the aorta are well known.   Hueper has preferred his own explanation of the intimal lesions, but to my mind his work fully confirms the theory of Leary.   The reticuloendothelial cells of the liver and other organs become engorged with these substances, and in the case of methyl cellulose (Hueper[57a]) the foam cells are readily demonstrated in blood smears and are found in the aortic intima. Surely the most reasonable explanation of their presence in the latter is that they have come from the blood stream by the simple process of passing through the endothelium.   It would be most interesting to know the specific gravity of the various substances Hueper used in the form in which they are present in the lipophages, in view of the hypothesis suggested earlier in this paper that relative lightness is the reason why these cells are the first to enter the marginal stream.

VALIDITY OF CHOLESTEROL FEEDING EXPERIMENTS

There has been a tendency of recent years to declare that the lesions caused in rabbits by feeding them on cholesterol has no relationship with human atherosclerosis.   Leary[58] has already dealt with most of these criticisms, but to end this paper a few extra points are discussed. The statement that the experimental lesion is confined to the rabbit is now out of date, since Steiner and Kendall[44] can produce it in dogs. The different distribution in the rabbit's aorta as compared with the

---

57. Hueper, W. C.:   (*a*) Am. J. Path. **18**:895, 1942; (*b*) **21**:1021, 1945; (*c*) Arch. Path. **39**:117, 1945; (*d*) **41**:130, 1946.
58. Leary, T.:   Arch. Path. **21**:459, 1936.

human aorta is not a valid ground of criticism, for in the human infant, an animal that is not normally vertical, the site is much the same as in the rabbit.   It is true that in the rabbits placed upright in jars by Wilens [59] the increased incidence of atheroma was not in the abdominal aorta, but the effect of posture in destroying the elasticity of the media of the lower part of the aorta is probably pronounced only after many years and not to be expected in the shorter experimental period, during most of which time the rabbits were asleep.   That hypercholesteremia has no relation to the human disease can be upheld only by ignoring the work of Rabinowitch [60] on diabetes and, above all, by neglecting consideration of hereditary xanthomatosis (Bloom and co-workers [61]; Boas and Adlersberg [62]).   In this disease high blood cholesterol is combined with extensive atherosclerosis and extraordinary susceptibility to coronary occlusion.   Page [42] and Weinhouse [63] have rightly pointed out that human atherosclerosis is not always associated with hypercholesteremia, but in this connection it is necessary to stress a point in Leary's theory that Leary himself does not emphasize enough, i. e., that the lesion is not directly caused by the high blood cholesterol but by circulating lipophages, over years, perhaps only a few cells at any one time. Granted that the common cause of the circulation of these lipophages is high blood cholesterol, is it necessary to assume that this is invariably the case?   Per contra, if there is hypercholesteremia, but for some reason the filling of the reticuloendothelial cells with lipids and their release from the liver are in abeyance, atheroma will be inhibited.   This seems to be the most probable explanation of the fact that although alcohol has the effect of increasing experimental hypercholesteremia, atheroma is reduced, for in these rabbits deposition of lipid in the liver is also lessened (Eberhard [64]).   Finally, it must be emphasized that the conceptions of the pure cholesterol school as enunciated by Leary and those of the school that insists on the primary influence of disease of the media (Duff [24]) are not or should not be opposed, the whole purport of this paper being that both are part of one and the same theory.   I do not claim to have made any original observations but have picked the brains of many authors and out of a welter of facts produced, it is hoped, a coherent and novel synthesis.   Probably the sole suggestion that has not been made by some one, somewhere, before, is that the lipophage, by virtue of its lightness, would be the first cell to come into contact with

59. Wilens, S. L.: Am. J. Path. **19**:293, 1943.
60. Rabinowitch, I. M.: Ann. Int. Med. **8**:1474, 1935.
61. Bloom, D.; Kaufman, S. R., and Stevens, R. A.: Arch. Dermat. & Syph. **45**:1, 1942.
62. Boas, E. P., and Adlersberg, D: J Mt. Sinai Hosp. **12**:84, 1945.
63. Weinhouse, S.: Arch. Path. **35**:438, 1943.
64. Eberhard, T. P.: Arch. Path. **21**:616, 1936.

the aortic endothelium and that, because of its relative inertness, it would be pushed into the intima by the blood pressure, and thus the main feature of atheroma would be formed.

SUMMARY AND CONCLUSIONS

The theory of Leary that atherosclerosis is caused by circulating lipophages is carried to its logical conclusion. It is pointed out that these cells, as all cells, would normally be in the center or axial current of the blood; that with slowing ·of the blood stream these cells would move into the peripheral or marginal stream and come into contact with the endothelium; that, because of their lightness, they would do so before any other cell, and then, because of their relative inertness, would be passively pushed into the intima by the blood pressure. Once inside the intima, the majority would be retained there by the internal elastic membranes which are impermeable to these fat cells. Atheroma is mainly a disease of the elastic arteries because, as they are filled only in systole and are partially emptied during the whole cardiac cycle, the current has a certain intermittency, which is less the case in other vessels that are farther from the heart. The disease also occurs in the elastic arteries because they dilate with age or with disease and thus the velocity of the blood in them is further reduced. The disease does not occur in other vessels because the current is less intermittent, the vis a tergo pushing the lipophage into the intima, i. e., the blood pressure, is less, and the internal elastic lamellas are either less well developed or do not exist. Much of the experimental work of the last few years is discussed in the light of this hypothesis.

DISTRIBUTION OF PARIETAL CELLS IN GASTRIC DISEASE

WILLIAM A. MEISSNER, M.D.
BOSTON

THERE is now general agreement that the source of the hydrochloric acid of the stomach is the parietal cells of the gastric mucosa.[1] Although the determination of gastric acidity has become almost a routine procedure in the study of stomach disorders, particularly in cases of peptic ulcer and cancer, the state of the cells which secrete the acid has been left largely to conjecture.

An attempt was made to determine whether there are quantitative or qualitative differences in the parietal cells in conditions in which there is usually hyperacidity (peptic ulcer) as contrasted with conditions in which the acid is usually low or absent (gastric cancer). The problem is of more than academic interest, since Hurst[2] and others have reported that the absence of acid found so frequently with gastric carcinoma usually, if not always, precedes the onset of the malignant process.

HISTOLOGY OF NORMAL PARIETAL CELLS

The parietal cells appear first in an embryo of 12 weeks, arising from undifferentiated cells about the necks of the gastric glands. In the adult stomach the parietal cells of each gastric gland are distributed throughout the entire length of the gland but are more common in the upper third (fig. 1). Their name "parietal" is derived from the fact that they are situated on the outer aspect of the cell layers of the gland and have no obvious contact with the lumen (fig. 2). They are often called "acid" or "oxyntic" cells. The cytoplasm stains readily with any of the acid dyes, such as eosin or phloxine, and can be shown to have small canaliculi and minute granules, which are related supposedly to secretory activity. The cells are oval or wedge shaped and average about 20 microns in diameter. The cytoplasmic outlines are distinct. The nuclei are frequently multiple (as many as eight have been found in an individual cell), small, round or oval, and often hyperchromatic. Although mitoses have been reported to occur, I have never observed them.

1. Babkin, B. P.: Secretory Mechanism of the Digestive Glands, New York, Paul B. Hoeber, Inc., 1944. Ivy, A. C.: Surgery **10**:861, 1941.

2. Hurst, A.: Lancet **2**:1023, 1929.

MATERIAL AND METHODS

Because at necropsy the stomach usually shows extensive autolysis, only specimens removed surgically were studied. Of the 200 examined, 81 had been resected for duodenal ulcer, 80 for gastric carcinoma and 39 for gastric ulcer. The specimens were placed in Zenker's solution soon after removal, often within an hour. Blocks were taken from the same representative areas in each specimen as far as possible and were designated by appropriate letters (fig. 3). Since relatively few of the 200
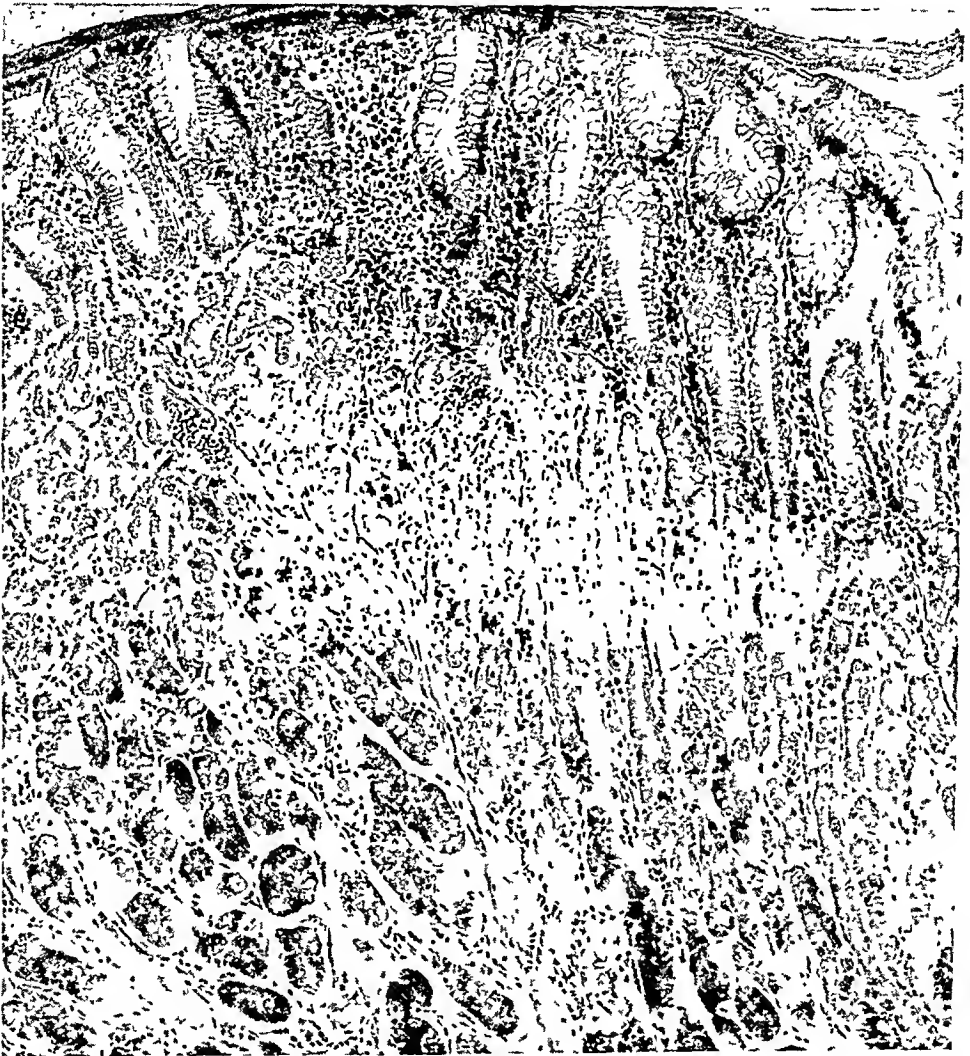
Fig. 1.—Abundance of parietal cells in a stomach resected because of duodenal ulcer. Eosin–methylene blue; × 100.

operations were total gastrectomies, the cardia of the stomach could not be adequately examined and is therefore not included in the study. All sections were stained with eosin–methylene blue.

An actual count of all the parietal cells in each of the blocks was too time consuming to be practicable. Therefore, a subjective estimate was made on each block as to whether the cells were abundant (fig. 1) or few (fig. 4). While there was a small amount of overlapping of the two groups, the great majority of micro-

scopic fields fell quite definitely into one or the other group. Such an estimate was made on each block.

In order to note qualitative changes in the parietal cells which might be seen in routine microscopic preparations, features such as size and shape of the cells, granularity or vacuolation of cytoplasm, density of nuclear material, size, shape and number of nuclei and distribution of cells in the gland were given particular attention.
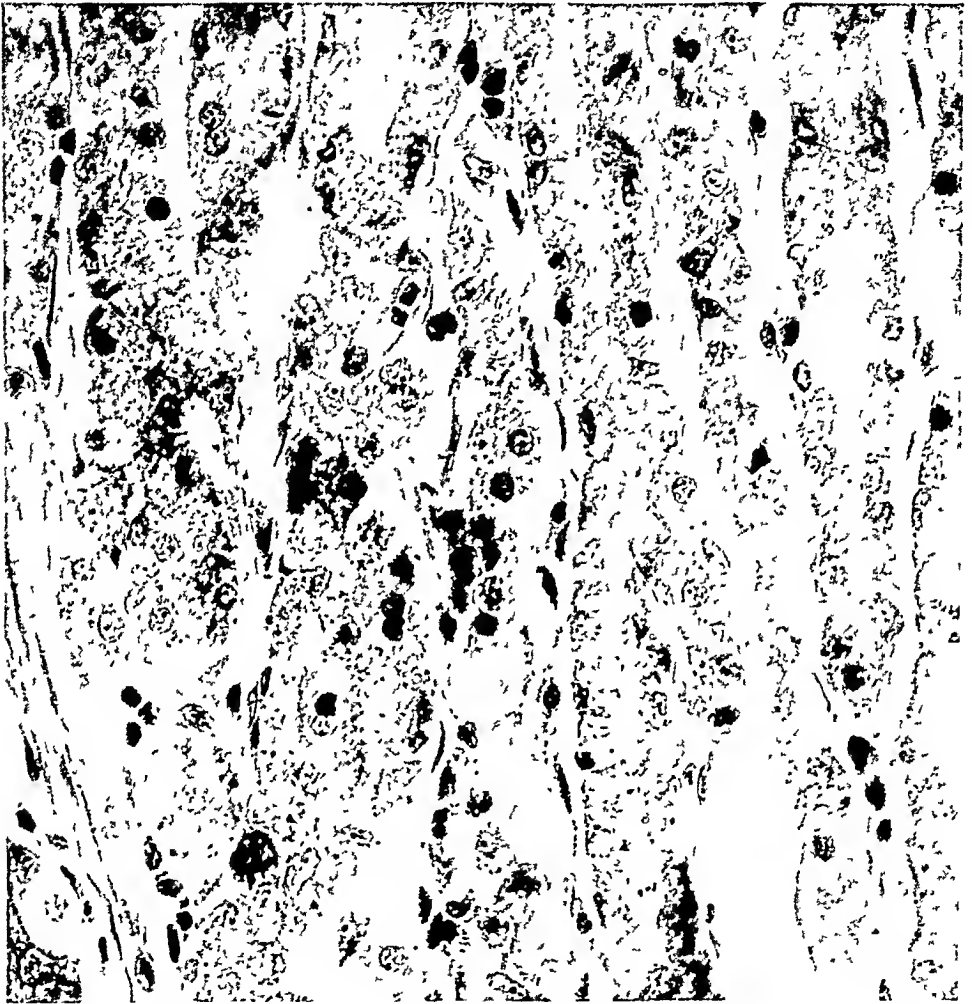
Fig. 2.—Higher power of a part of the section shown in figure 1.   × 400.

RESULTS

The great majority of specimens were from patients 40 to 60 years of age. The ages of the patients with duodenal ulcer averaged 47 years; those of the patients with gastric ulcer, 51 years, and those of the patients with carcinoma, 55 years. Males predominated in a 5 to 1 ratio.

In all specimens the parietal cells diminished in number as the pylorus was approached. They were likewise somewhat fewer along
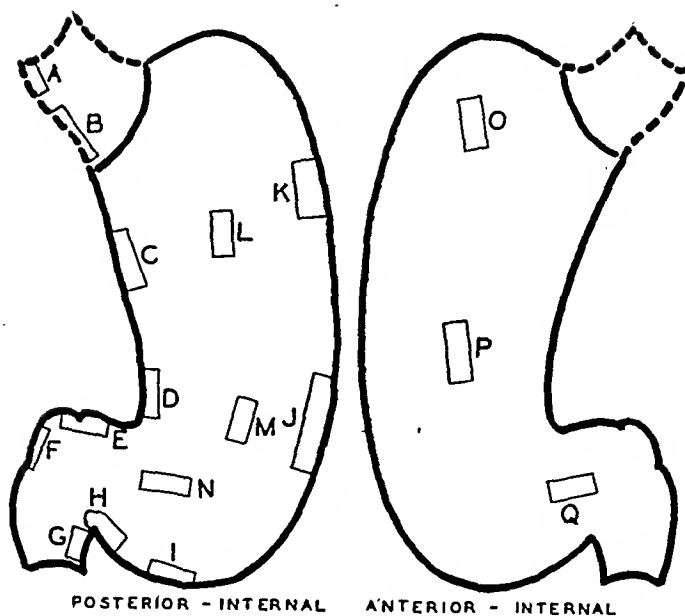
Fig. 3.—Chart showing areas from which blocks were taken for microscopic study.

Fig. 4.—Photomicrograph of a section showing few parietal cells. Eosin–methylene blue; × 100.

the entire lesser curvature as contrasted with opposite areas on the walls or greater curvature. This distribution corresponds in general with that found by Berger [3] in 8 normal stomachs in which he did actual counts of the parietal cells.

Quantitative Variations in Ulcer and Cancer.—It was found that the stomach could be divided roughly into four zones: the fundus, the body, the pyloric antrum and the pyloric canal. Except for the slight diminution of the number of parietal cells on the lesser curvature, any section of a zone fairly accurately represented the distribution of the parietal cells of that entire zone of any given specimen (fig. 5). To simplify the comparison of parietal cells in ulcer and cancer, only one block from each

POSTERIOR - INTERNAL   ANTERIOR - INTERNAL
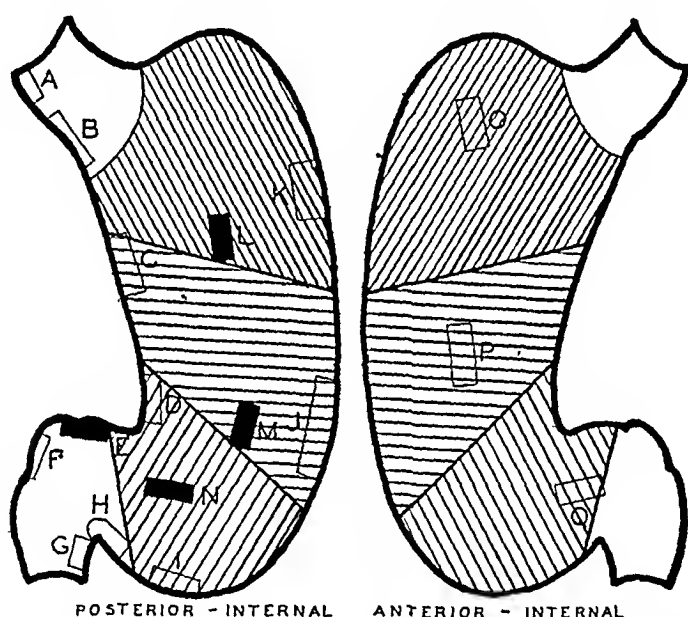
Fig. 5.—Chart showing the stomach divided into four zones for purposes of comparison.

zone needs to be considered. Sections E, N, M and L may be taken to represent the zones in which they lie, respectively.

The pyloric canal and the pyloric antrum showed few parietal cells in the great majority of cases of ulcer and of cancer (fig. 6). Furthermore, there was no significant difference in the percentage of cases showing this diminution between ulcer and cancer.

In regard to the body and the fundus, however, particularly in regard to the latter, there was a significant difference in the percentage of cases of ulcer and of cancer which showed few parietal cells; 54 per cent of the cases of cancer showed a small number of parietal cells

3. Berger, E. H.: Am. J. Anat. **54**:87, 1934.

in the fundus, while only 30 per cent of the cases of gastric ulcer and 10 per cent of the cases of duodenal ulcer showed such diminution.

There is, then, a tendency for cases of cancer to show fewer parietal cells than cases of ulcer, especially if the ulcer is in the duodenum. However, it can be readily seen from the graph (fig. 6) that a diminution of the number of parietal cells was not a constant finding in cases of cancer. Many stomachs with complete achlorhydria showed abundant parietal cells (fig. 7), and none of the cases of cancer showed complete absence of such cells.

Qualitative Changes.—As far as qualitative changes occurring in the individual parietal cell are concerned, as seen in routine stains there were no nuclear or cytoplasmic alterations which could be correlated with the presence or the absence of either peptic ulcer or gastric cancer. Cyto-
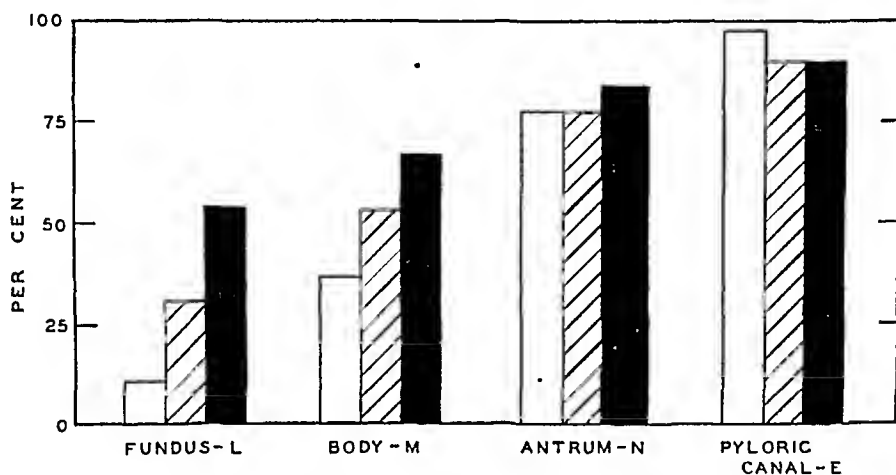
Fig. 6.—Graph showing percentage of sections with diminished parietal cells in cases of ulcer and of cancer. White columns represent duodenal ulcer; cross-hatched columns, gastric ulcer; black columns, carcinoma.

plasmic granules and vacuoles, possible precursors of the acid secretion, were frequently found associated with both types of gastric lesion.

COMMENT

According to Best and Taylor,[4] 60 per cent of cases of gastric carcinoma show complete absence of acid. It is somewhat surprising, then, that the acid-forming cells do not show fairly constant numerical or morphologic changes in cancer. Since the only change observed was a tendency for the parietal cells to be fewer and since abundant parietal cells were at times found with cancer and complete anacidity, one must look further for explanations of the problem.

4. Best, C. H., and Taylor, N. B.: The Physiological Basis of Medical Practice, ed. 4, Baltimore, Williams & Wilkins Company, 1945.

Numerous theories have been proposed to explain diminished gastric secretion in the presence of cancer. One of the more recent is that of Brunschwig,[5] who suggested that the cancer forms a "secretory depressant." Other explanations which have been offered include neutralization of gastric juice or suppression of secretion as a result of the action of
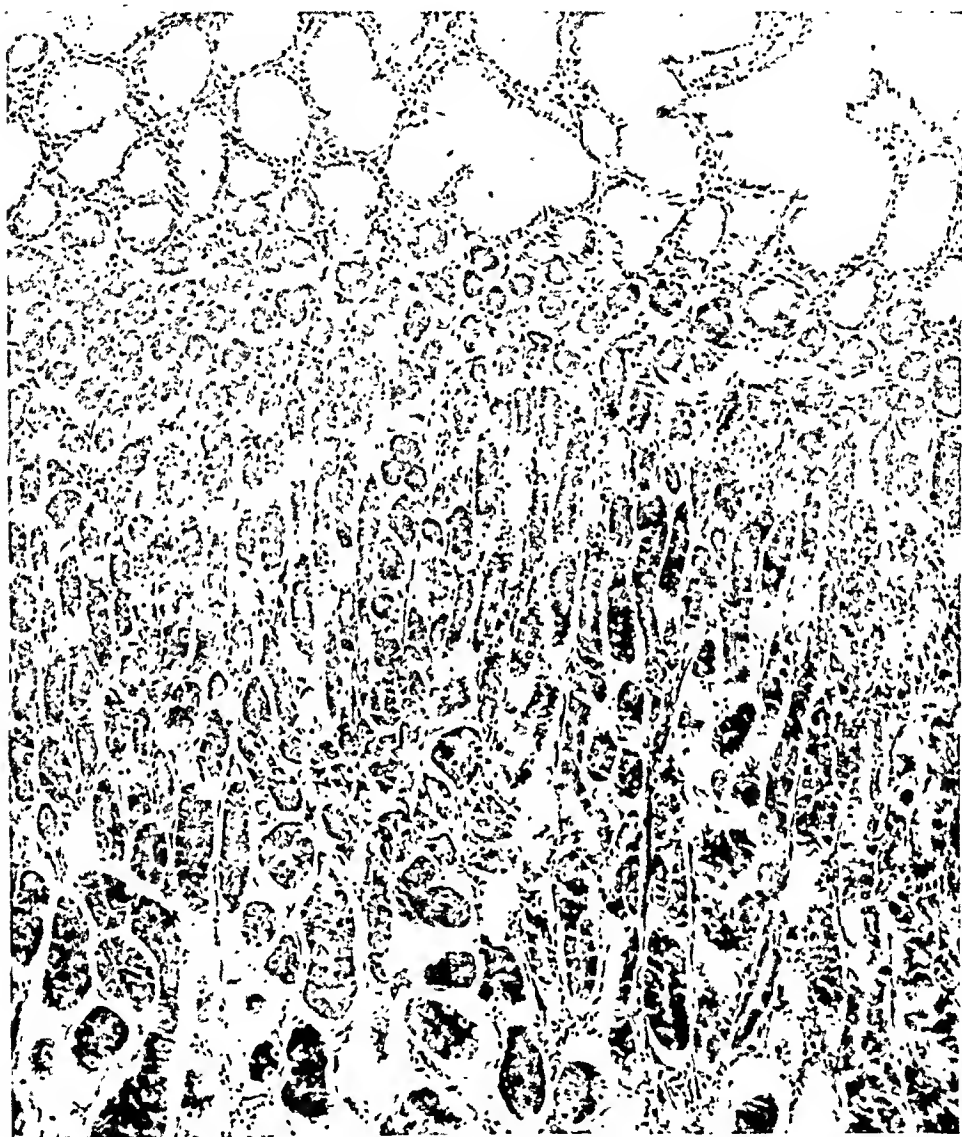
Fig. 7.—Abundant parietal cells in a stomach with cancer and with complete achlorhydria. Eosin–methylene blue; × 100.

the duodenal contents or mucus, interference with gastric nerves, chloride starvation and chronic gastritis. Polland and Bloomfield [6]

5. Brunschwig, A., and others: Surg., Gynec. & Obst. 70:25, 1940.

6. Polland, W. S., and Bloomfield, A. L.: Bull. Johns Hopkins Hosp. 46:307, 1930.

reviewed such explanations and concluded that "one can hardly evade the supposition that . . . gastritis may be the direct cause of the defective secretion."

This is a view held by numerous observers and one which is usually reached by a process of exclusion rather than by positive findings. It is true that it has been shown [7] that the incidence of chronic gastritis is higher in the fundus and the body of the stomach in cancer than it is in ulcer, and it is easy to assume that the diminution of the number of parietal cells which was found may be related to chronic gastritis. However, there is still left the difficulty of explaining anacidity in the presence of numerous parietal cells and in the absence of gastritis. Moreover, gastritis is a common condition and may be associated with hyperacidity as well as with diminished acid secretion.

It would seem, then, that there are probably several methods of bringing about the diminished secretion of acid which is seen in the presence of cancer of the stomach and that further investigation is warranted to determine more clearly the causes of this phenomenon and its relationship to the development of gastric cancer.

SUMMARY AND CONCLUSIONS

A series of 200 stomachs surgically resected for gastric carcinoma or for peptic ulcer of the stomach or the duodenum was examined with particular reference to qualitative and quantitative changes in the parietal cells in the different pathologic states.

The number of parietal cells diminishes as the pylorus is approached and is somewhat less along the lesser curvature than in corresponding areas on the walls or greater curvature.

The only quantitative change of significance was that in many cases of carcinoma there was a diminution of the number of parietal cells in the body and the fundus of the stomach, whereas in cases of peptic ulcer, especially cases of duodenal ulcer, such a diminution was less frequent.

A reduction of the number of parietal cells was not a constant finding in cases of cancer of the stomach; in many cases of cancer and complete anacidity there was an abundance of parietal cells. No stomach showed complete absence of such cells.

As seen in routine stains, there were no qualitative changes in individual parietal cells which could be correlated with ulcer or with cancer.

---

7. Meissner, W. A.: J. Nat. Cancer Inst. **5**:377, 1945.

SOME FACTORS INFLUENCING BROWN · DEGENERATION OF THE ADRENAL GLAND IN THE SWISS ALBINO MOUSE

CHARLES E. TOBIN, Ph.D.

AND

JEAN P. BIRNBAUM, B.A.

ROCHESTER, N. Y.

THE TERM "brown degeneration of the adrenal gland of the mouse" has been used with reference to the pigment found mainly in the cells of the inner cortical zones, which appears brownish yellow in unstained sections as well as in those stained with hematoxylin and eosin. The pigmented cells usually form at the corticomedullary junction, but they may extend into the peripheral zones of the cortex, as well as among the cells of the medulla. Although brown degeneration of the adrenal gland of the mouse has been reported to occur under various conditions, its mode of origin is not well understood, for it is found mainly in certain strains of mice,[1] occurs most frequently in old animals,[2] is not correlated with the incidence of neoplasms in different strains of mice,[1a, b] and may be produced by administration of estrogen.[3]

Since the pigment of brown degeneration· appears to be of a lipoid nature from its reactions with osmic acid and sudan stains (Cramer and Horning [2b]), the following experiments were carried out to determine whether qualitative and quantitative differences of the fat content of the diet would· influence the incidence of brown degeneration in a strain of mice which normally has only a slight amount of this pigment in the adrenal glands when fed a commercial diet containing a relatively

1. (a) Kreyberg, L., and Ecker, R.: Avhand, utgitt av det Norsk. Vidensk. Akad. i. Oslo. I. Mat. Nat. Kl. **3**:1, 1939. (b) Blaisdell, J. S.; Gardner, W. U., and Strong, L. C.: Cancer Research **1**:283, 1941. (c) Dobrovolskaïa-Zavadskaïa, N., and Zéphiroff, P.: Compt. rend. Soc. de biol. **128**:971, 1938.

2. (a) Burrows, H.: J. Path. & Bact. **43**:121, 1936. (b) Cramer, W., and Horning, E. S.: ibid. **44**:633, 1937. (c) Blaisdell, Gardner and Strong.[1b]

3. (a) Lacassagne, A., and Raynaud, A.: Compt. rend. Soc. de biol. **124**:1183, 1937. (b) Burrows.[2a] (c) Cramer and Horning.[2b]

small quantity of fat. Some of the diets were also supplemented with tocopherols (vitamin E) to test whether the latter have an influence on the formation of this pigment. In view of the known changes of the adrenal glands of mice treated with estrogenic substances and the influence of these on fat storage in rats fed a diet rich in fat (Loeb [4]), the effect of supplementary injections of estrogen was also studied.

MATERIALS AND METHODS

Male and female mice of a homogeneous strain (Swiss albino) were used. The animals of the breeding colony were fed a commercial diet [5] and given tap water ad libitum. In most instances mothers and their young were started on the experimental diets on the day of delivery. In the groups given an estrogen, all members of each litter received subcutaneous injections of estradiol benzoate [6] in sesame oil (1 mg. per cubic centimeter) once weekly, doses of 0.01, 0.03 or 0.05 cc. being given for the first three weeks, and doses of 0.1 cc. during the subsequent weeks.

Approximately equal numbers of males and females of each age group were killed with illuminating gas and their adrenal glands fixed in Zenker's solution to which glacial acetic acid had been added to the concentration of 5 per cent. Paraffin sections, cut at 8 microns through the center of these glands, were stained with Verhoeff's carbolfuchsin for one hour at 65 C., destained with acid alcohol (3 per cent of hydrochloric acid in 80 per cent alcohol) for fifteen minutes and counterstained with Ehrlich's hematoxylin. Adjacent sections were stained with Ehrlich's hematoxylin and eosin; osmic acid, sudan IV, sudan black B, or nile blue sulfate for fats; by the Turnbull blue method for iron, or with Schultz cholesterol reagents.[7] The fluorescence of this pigment was observed in paraffin sections under ultraviolet rays. Adrenal glands (from selected animals) fixed in Levi's or Flemming's osmic acid were sectioned in paraffin and couterstained with Ehrlich's hematoxylin. Frozen sections of adrenal glands fixed in 10 per cent neutral solution of formaldehyde U. S. P. and cut at 15 microns were stained with osmic acid, sudan IV, nile blue sulfate, carbolfuchsin or Schultz cholesterol reagents. Other tissues from these experimental animals, prepared by these methods, will be described in a separate report.

4. Loeb, H. G.: Proc. Soc. Exper. Biol. & Med. **51**:330, 1942.

5. The diet is marketed under the name "Wayne dog blox." See table 1 for composition.

6. The alpha-estradiol benzoate was supplied by Ciba Pharmaceutical Products, Inc., Summit, N. J.

7. The following method was used to stain paraffin sections with sudan IV or sudan black B: Remove paraffin and hydrate sections to 70 per cent alcohol; cover sections with sudan IV (acetone-alcohol solution of Herxheimer) for two to five minutes or with sudan black B (a filtered saturated solution in equal parts of 70 per cent alcohol and glycerin) for thirty minutes; after use of either stain, wash in 70 per cent alcohol; counterstain if desired but avoid higher percentages of alcohol, which will remove the stain; hydrate, apply glycerin-gelatin (equal parts of glycerin and gelatin) and a cover glass. For the Schultz reaction sections are prepared as follows: Remove paraffin, hydrate and place sections in a 2.5 per cent aqueous solution of violet ferrous alum at 37 C. (or it can be used cold) for two to six hours, blot dry, add a few drops of equal parts glacial acetic and concentrated sulfuric acids to sections and apply a cover glass.

GENERAL STAINING REACTIONS OF THE PIGMENT
OF BROWN DEGENERATION

Unstained Sections.—In sections of fresh or fixed (solution of formaldehyde U. S. P. or Zenker's) adrenal glands this pigment appeared brownish yellow and exhibited a similarly colored fluorescence under ultraviolet rays.

Stained Sections.—In frozen sections this pigment stained black with osmic acid, blue or light purple with nile blue sulfate, yellowish red with sudan IV, greenish blue with Schultz cholesterol reagents and bright red with carbolfuchsin. It usually stained much more intensely by these methods than did the lipids normally present in cortical cells.

In paraffin sections of adrenal glands fixed in Zenker's solution as described, this pigment stained yellowish brown with hematoxylin and eosin, yellowish red with sudan IV, black with sudan black, grayish black with osmic acid, blue or purple with nile blue sulfate, greenish blue with Schultz reagents and bright red with carbolfuchsin. Since cortical cells devoid of pigment did not stain with sudan IV or sudan black, osmic acid, nile blue sulfate or Schultz cholesterol reagents, this pigment, unlike the fat in the normal cortical cells, was insoluble in the usual dehydrating and clearing agents. After carbolfuchsin staining the pigment granules or globules were more distinctly depicted than after hematoxylin and eosin, osmic acid, sudan IV or sudan black staining. This pigment invariably gave a negative reaction for iron (Turnbull blue reaction). In tissue fixed in Levi's or Flemming's osmic acid solutions, the pigment stained black, the nonpigmented cortical cells were stained gray where the cells contained little lipoid material or black where lipoid material was abundant, and X zone cells undergoing degeneration were also stained black.

CLASSIFICATIONS OF THE STAGES OF BROWN DEGENERATION

The classification of the stages of brown degeneration proposed by Cramer and Horning,[8] subsequently used by Blaisdell, Gardner and Strong [1b] and others investigating this problem, was based primarily on studies of sections of the adrenal glands of relatively old animals stained with osmic acid, sudan III, or hematoxylin and eosin. This classification did not consider the pigment formed in the adrenal glands of younger animals where the X zone was present, or the condition of the cells during the progressive development of the pigment in different zones of the adrenal gland. Furthermore, the stains used did not depict the pigment as clearly as the carbolfuchsin (acid-fast) method used in this study.

---

8. Cramer, W., and Horning, E. S.: Am. J. Cancer **37**:343, 1939.

The acid-fast and other staining reactions of the adrenal glands of young and old animals in our experimental and control series have led to the adoption of a classification of the stages of brown degeneration based on two major criteria: (1) the sequence and the relative extent
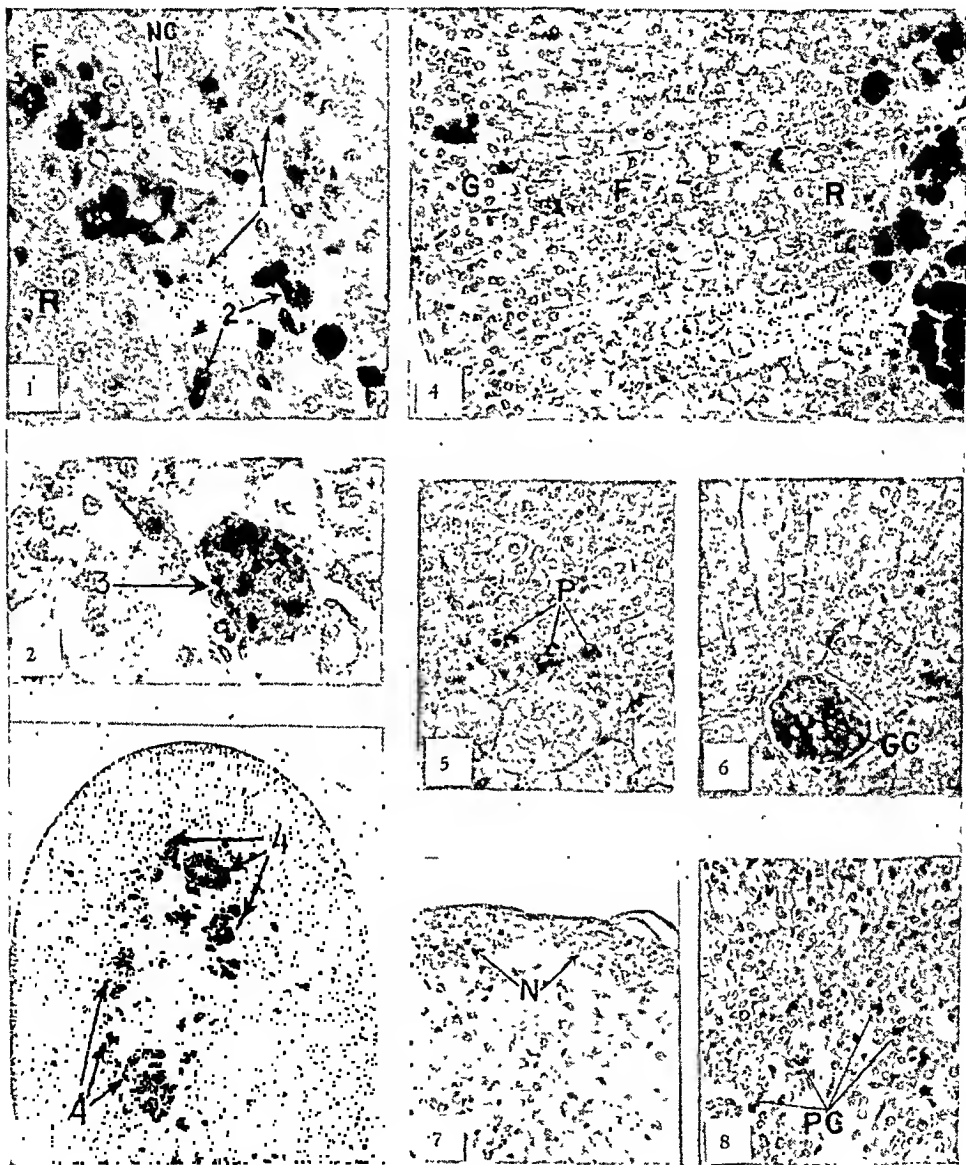
Figures 1 to 8
(See legend on opposite page)

of the pigment deposition within cortical cells, as well as the cytologic changes of these cells (qualitative), and (2) an estimate of the gross amount of this pigment in the whole adrenal gland based on representative sections of the gland (quantitative).

Qualitative Stages.—These stages are designated 0, 1, 2, 3 and 4. In stage 0 no pigment is present in any cells of the adrenal gland. In stage 1 granules of pigment appear in the cytoplasm of normal-appearing cells of the inner fascicular, the reticular or the X zone (fig. 1). In stage 2 pigment in the form of larger granules or globules, irregular in size, fills most of the cytoplasm in isolated cells of the reticular or the X zone, which have eccentrically located, pyknotic nuclei but intact cell membranes (fig. 1). In stage 3 multinucleated giant cells filled with pigment (fig. 2) are present in the reticular or the X zone, and the stage 1 and 2 cells appear in the glomerular and fascicular zones. In stage 4 a prominent ringlike area of pigmented cells forms at the corticomedullary junction, composed mainly of stage 2 and 3 cells. Cells of stage 1 were also found in other parts of the cortex, and if the X zone was absent, pigmented cells of various stages were found in the medulla (figs. 3 and 4).

Quantitative Estimate.—The total amount of pigment found in representative sections through the cortex and the medulla has been expressed as a graded series from no pigment (0) to the greatest amount of pigment observed (IV). This quantitative estimate appeared to be a necessary adjunct of the determination of the qualitative stages, since in any

---

EXPLANATION OF FIGURES 1 TO 8

Photomicrographs of paraffin sections of adrenal glands stained with carbol-fuchsin (acid-fast) and Ehrlich's hematoxylin.

Fig. 1.—Inner fascicular (*F*) and reticular (*R*) zones of the adrenal cortex from a 217 day old male fed the high lard diet during the last one hundred days, showing normal-appearing cells (*NC*), stage 1 cells (*1*) containing granules or globules of acid-fast pigment but otherwise appearing normal, and stage 2 cells (*2*) with increased amounts of acid-fast pigment and pyknotic nuclei ($\times$ 196).

Fig. 2.—Reticular zone of the adrenal cortex from a 253 day old male fed the high lard diet for the last two hundred days. A stage 3 giant cell (*3*), containing several nuclei and large amounts of pigment, and several stage 1 cells with peripheral granules or globules of pigment are shown ($\times$ 196).

Fig. 3.—Adrenal gland from a 275 day old male fed the high lard diet for the last two hundred and fifty-four days. This shows a stage 4 reaction with a ringlike area of pigment (*4*) at the corticomedullary junction and various stages of pigment formation in other parts of the cortex and the medulla; the X zone had degenerated ($\times$ 19.5).

Fig. 4.—Zones of the cortex of the adrenal gland shown in figure 3. The pigmentation of cells of the glomerular (*G*), fascicular (*F*) and reticular (*R*) zones is shown ($\times$ 91.5).

Fig. 5.—Reticular zone of the cortex (above) and the medulla (below) of the adrenal gland of a male 200 days of age which was fed the stock diet, showing the usual amount of pigment (*P*) found in older animals ($\times$ 91.5).

Fig. 6.—Similar area from a 200 day old male showing the maximum amount of pigment found in adrenal glands of older animals fed the stock diet. One of three widely separated giant cells (*GC*) containing pigment is shown in this section at the corticomedullary junction ($\times$ 91.5).

Fig. 7.—Part of the adrenal cortex from a male fed the stock diet and given injections of an estrogen over a period of one hundred and fifty days. Note the nodular-like formations (*N*) containing pigment in the glomerular area ($\times$ 91.5).

Fig. 8.—Inner part of the adrenal cortex from a 10 day old male whose mother was fed the high lard diet during the last quarter of gestation and lactation. Pigmented cells (*PC*) are present in this area ($\times$ 91.5).

given qualitative stage the number of cells may not be the same in adrenal sections from different experimental groups and therefore alone may not be an index of the total pigment formation.

These two series of symbols representing the qualitative and the quantitative differences of .pigmentation are used in table 2 to summarize the observations made on the adrenal glands in the various experimental groups to be discussed.

EFFECT OF DIETARY FAT, OF VITAMIN SUPPLEMENTS AND OF ESTROGEN ON BROWN DEGENERATION

The components of the diets used are shown in table 1. The low fat and the hydrogenated cottonseed oil ("crisco") diet were supplemented with vitamins A and D.[9]

Dietary Fat.—The adrenal glands of animals fed the stock diet, containing 4 per cent fat (iodine number: 86), had no pigment at thirty

TABLE 1.—*Diets*

		Experimental Diets		
Stock Diet,* per Cent		Low Fat,† per Cent	High Lard,† per Cent	"Crisco," per Cent
Protein....................22.0	Casein.............	20.0	20.0	20.0
Carbohydrate...............40.0	Starch.............	70.0	50.0	50.0
Salts......................... 6.4	Salts..............	2.5	2.5	2.5
Fiber........................ 5.0	Yeast.............	7.5	7.5	7.5
Moisture....................22.6	Lard..............	...	18.0	...
Fat.......................... 4.0	Cod liver oil.......	...	2.0	...
	"Crisco"...........	...	...	20.0
	Vitamin A‡........	20 U.S.P. units	...	20 U.S.P. units
	Vitamin D‡........	2 U.S.P. units	...	2 U.S.P. units

* The stock diet consisted of "Wayne dog blox." The manufacturer's analysis is given here.
† The diet was deficient in tocopherols.
‡ Commercial concentrate of vitamins A and D was fed orally twice weekly.

days and relatively small amounts at the other age periods. With the exception of a few isolated pigment-containing multinucleated giant cells in the reticular zone of the glands of 4 animals over 200 days of age, stage 1 or 2 cells were the usual types observed (figs. 5 and 6). Mice fed the low fat diet, deficient in tocopherols and containing less than 1 per cent fat,[10] had no pigment in their adrenal glands at the various age periods. However, those fed the high lard diet, deficient in tocopherols but containing 18 per cent lard and 2 per cent cod liver oil (iodine numbers: 66 and 173, respectively), had considerably more

9. Vitamins A and D (concentrated), supplied by Distillation Products, Inc., Rochester, N. Y., were given orally twice a week as 20 U. S. P. units of A and 2 U. S. P. units of D.

10: C. G. Mackenzie, J. B. Mackenzie and E. V. McCollum (Biochem, J. **33**: 935, 1939) have shown that unextracted yeast and casein may contain up to 5.8 and 1.5 per cent lipid, respectively, and therefore this diet should be designated as low in fat rather than fat free.

pigment at each age period than did animals fed the stock diet. Diets comparable to the diet just mentioned or others containing a higher percentage of cod liver oil have been reported as causing pigment to be deposited in certain tissues of rats [11] which resembles in its staining reactions this pigment deposited in the adrenal glands of mice.

Evidence that pigmentation of adipose and other tissues of rats is due to the presence of unsaturated fatty acids in tocopherol-deficient diets [11e] and that an adequate supply of tocopherols prevents the pigmentation due to this dietary factor [12] suggested a study of the effects of such fats, with and without supplementary tocopherols, on the adrenal pigment of mice.

The highly unsaturated fraction of the fatty acids from cod liver oil (iodine number: 319), prepared according to the procedure followed by Dam and Granados,[11e] when fed at 3 per cent and 1 per cent levels in the low fat diet, replacing equivalent amounts of starch, produced extensive adrenal pigmentation. Retardation of growth was quite definite at the higher level but was only slight at the lower level of feeding.

In view of the observed influence of the dietary unsaturated fatty acids of tocopherol-deficient diets on adrenal pigmentation, a relatively saturated, hydrogenated vegetable oil ("crisco"—iodine number: 75) was substituted for the lard and the cod liver oil of the high lard diet. No pigment was observed in the adrenal glands of animals fed this diet or fed this diet supplemented with 1 per cent of the unsaturated fatty acid fractions of cod liver oil—so effective in causing pigmentation when incorporated in the low fat diet. That the protective action of the "crisco" diet was due to its tocopherol content [13] rather than to the reduction effected in unsaturation of the dietary fat is indicated by experiment in which tocopherols were given in conjunction with the high lard diet (see next section).

Tocopherols.—Animals fed the high lard diet had little pigment in their adrenal glands when given 10 to 15 mg. of tocopherols [14] orally once a week or when the tocopherols were incorporated in this diet to a concentration of 1 per cent. This dosage of tocopherols was more than adequate for normal reproduction of animals fed the high lard diet. Increasing the oral dose of tocopherols to 20 mg., given once a week, or the content in the diet to 3 per cent, resulted in complete absence of

11. (*a*) Martin, A. J. P., and Moore, T.: J. Hyg. **39**:643, 1939. (*b*) Mason, K. E., and Emmel, A.: Yale J. Biol. & Med. **17**:189, 1944; (*c*) Anat. Rec. **92**:33, 1945. (*d*) Mason, K. E.; Dam, H., and Granados, H.: ibid. **94**:265, 1946. (*e*) Dam, H., and Granados, H.: Science **102**:327, 1945; (*f*) Acta physiol. Scandinav. **10**:162, 1945.

12. Mason and Emmel.[11b] Mason, Dam and Granados.[11d] Dam and Granados.[11f]

13. Hydrogenated cottonseed oil and other vegetable oils usually contain 1 to 2 mg. of tocopherol per gram of oil (P. L. Harris, personal communication).

14. The concentrated mixture of natural tocopherols (approximately 54 per cent tocopherols) was supplied by Distillation Products, Inc., Rochester, N. Y.

adrenal pigment. Four males and 4 females (data not included in table 2) fed the high lard diet for thirty days and then given the higher dose of tocopherols with this diet over a period of one hundred and twenty days had no greater pigmentation of their adrenal glands than mice fed
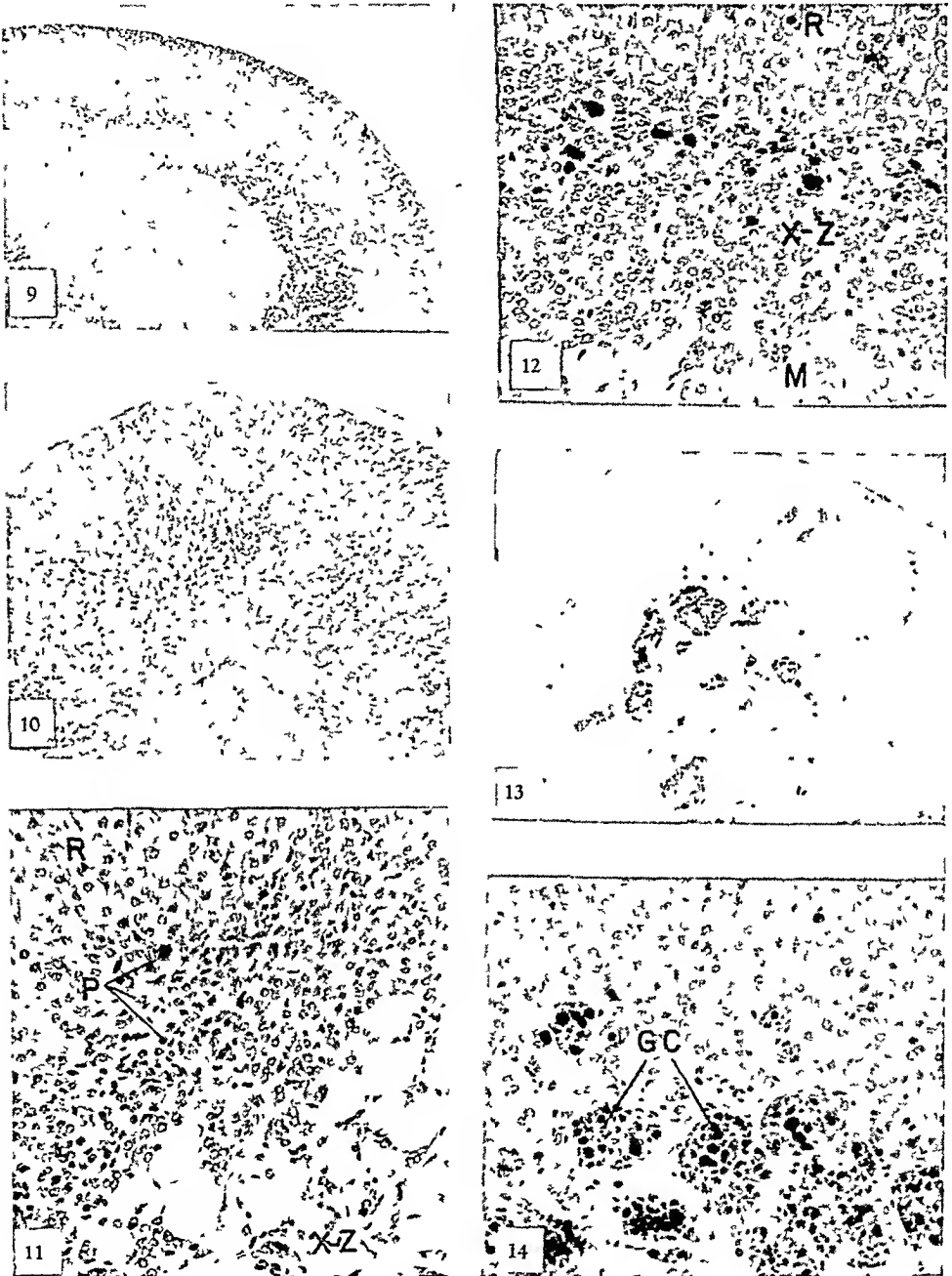
Figures 9 to 14
(See legend on opposite page)

this diet for only thirty days, indicating that deposition of pigment may be inhibited at any time by tocopherol feeding.

Animals fed the low fat diet containing 1 per cent fatty acids from cod liver oil and given 20 mg. of tocopherols orally once a week did have pigment in their adrenal glands. This may be attributed to the fact that such animals were receiving approximately 50 per cent more unsaturated fatty acids from cod liver oil than those animals fed the high lard diet.

Vitamins A and D.—These vitamins added to the low fat or to the "crisco" diet had no effect on adrenal pigment. The effect of their absence from the low fat diet was not studied. However, neither adrenal pigmentation nor any external sign of vitamin A deficiency was observed in 15 animals fed the "crisco" diet without supplemental vitamin A for one hundred and fifty days (data not included in table 2), although epithelial changes characteristic of this vitamin deficiency in the mouse [15] were observed in the vagina, the seminal vesicles, the testes and other organs. The epithelial metaplasia of the urogenital system appeared to be enhanced when animals restricted to this regimen were given injections of estrogen, causing early urinary obstruction and death. The effects of vitamin D deficiency were not studied.

Estrogen.—Since estrogen is known to produce brown degeneration in the adrenal glands, weekly subcutaneous injections of estradiol benzoate in sesame oil were given to animals fed the various diets. In mice fed on the high lard diet (with or without tocopherol supplementation)

EXPLANATION OF FIGURES 9 TO 14

Photomicrographs of paraffin sections of adrenal glands—all stained with carbol-fuchsin (acid-fast) and Ehrlich's hematoxylin except for that shown in figure 13, which was stained with Schultz cholesterol reagents.

Fig. 9.—Part of the adrenal cortex and medulla from a 75 day old virgin female fed the high lard diet and given adequate tocopherol therapy orally twice a week. The therapy started at 30 days of age. Pigment is present in the inner reticular and the X zone. However, less pigment was present than was found in adrenal glands of animals fed this diet for a comparable period without tocopherol therapy ($\times$ 20.5).

Fig. 10.—Part of the adrenal cortex and medulla from a 62 day old female fed the high lard diet and given injections of an estrogen once a week. The X zone is well preserved. Note the diffuse· distribution of pigmented cells throughout the cortex; pigment appearing to be in the medulla was in an intramedullary extension of a cord of X zone cells ($\times$ 20.5).

Fig. 11.—Part of the zona reticularis (R) and the degenerating X zone (X-Z) · from a 125 day old female fed the high lard diet during the last fifty days. Pigment (P) is present in cells of the inner part of the reticular zone but not in the vacuolated, degenerating cells of the X zone ($\times$ 96).

Fig. 12.—Part of the reticular zone (R), the X zone (X-Z) and the medulla (M) from the adrenal gland shown in figure 9. Pigment is present in the reticular as well as in the X zone ($\times$ 96).

Fig. 13.—Section, adjacent to the one shown in figure 3, treated with Schultz cholesterol reagents. The areas of brown degeneration show a positive reaction with the Schultz reagents, whereas the normal lipid-containing cells are negative—the lipoid material of the latter was dissolved by the chemicals used in the paraffin-embedding technic ($\times$ 27.5).

Fig. 14.—Section through part of the corticomedullary junction from the adrenal gland of a male 217 days old which was fed the high lard diet during the last fifty days ($\times$ 96). The acid-fast pigment does not uniformly fill the cytoplasm of the giant cells (GC). When this diet was fed for longer periods, the giant cells contained more acid-fast pigment. Compare with the pigment in the giant cells shown in figures 2 and 3.

the injected estrogen exerted little or no effect on adrenal pigmentation. On the other hand, estrogen appeared to augment this process in mice fed the stock diet and to cause adrenal pigmentation, which would otherwise not have occurred, in those fed the low fat or the "crisco" diet. These injections resulted in persistence of the X zone in both sexes, whereas normally this zone was absent from the adrenal glands of males at 30 days of age and those of virgin females at 90 to 125 days of age in this strain. Nodules containing pigment were found in the glomerular zone of the adrenal gland after estrogen had been administered for one hundred days or more. (fig. 7).

Cholesterol.—In view of the positive Schultz cholesterol reaction found in areas of brown degeneration in paraffin (fig. 13) and frozen sections of adrenal glands, 1 per cent crystalline cholesterol was added

TABLE 2.—*Brown Degeneration in Adrenal Glands of Mice*

Basic Diet	Dietary Supplements and Treatment	Animals		Highest Qualitative Stage * and Estimated Quantity at Given Days of Age			
		Males	Fe-males	30	50	100	>150
Stock diet	....	31	15	0-0	2-I	2-I	3-I
	Estrogen.......................	8	8	1-I	2-II	2-II	3-II
Low fat diet	....	8	13	0-0	0-0	0-0	0-0
	Fatty acids, 3%.................	11	10	3-II	3-III		
	Fatty acids, 1%.................	6	6	2-II	3-III	4-III	
	Tocopherols,† fatty acids, 1%..	4	4	2-II	3-II		
	Estrogen.......................	6	8	2-I	2-II	4-II	
High lard diet	....	18	24	2-II	4-III	4-IV	4-IV
	Tocopherols ‡...................	6	6	1-I	2-I	2-I	
	Tocopherols §...................	6	4	0-0	0-0		
	Estrogen.......................	10	6	1-II	3-III	4-IV	
"Crisco" diet	....	14	13	0-0	0-0	0-0	0-0
	Fatty acids, 1%.................	4	4	0-0	0-0		
	Estrogen.......................	9	4	2-I	2-I		

* The qualitative stage is indicated by the arabic, the quantitative estimate by the Roman numeral.
† Tocopherols were given orally once a week; dose, 20 mg.
‡ From 10 to 15 mg. of tocopherols was given orally once a week, or they were added to the diet to a concentration of 1 per cent.
§ Twenty milligrams of tocopherols was given orally once a week, or they were added to the diet to a concentration of 3 per cent.

to: the stock diet (pulverized), the high lard diet or the "crisco" diet (replacing equivalent percentages of lard and "crisco"). This cholesterol supplement increased but slightly the deposition of pigment in the adrenal glands when added to the stock diet (4 males and 5 females) for thirty to one hundred and fifty days, did not increase the pigmentation usually found with the high lard diet (10 males and 5 females) and produced no pigmentation of the adrenal glands when added to the "crisco" diet (10 males and 7 females). These data are not included in table 2. In all three groups the liver cells contained large deposits of fat which did not stain with carbolfuchsin in paraffin sections but did stain black with Levi's or Flemming's osmic acid fixation and red with sudan IV in frozen sections.

15. Wolfe, J. M., and Salter, H. P.: J. Nutrition 4:185. 1931.

COMMENT

From its staining reactions, the pigment of brown degeneration appeared to be of a lipoid nature. Yet the reaction to various fat stains (in paraffin sections) indicates that it, unlike the lipids in cells of the cortical parenchyma, was not soluble in the usual fat solvents. The reaction to nile blue sulfate suggests the presence of fatty acids.[16] However, this reaction, like that of osmic acid, sudan IV or sudan black, is not a specific histochemical test. The Schultz reaction usually indicates the presence of some form of cholesterol,[17] but it, too, should be accepted with caution, as indicated by Everett [18] from studies on the corpus luteum of the rat. If cholesterol constitutes a component of this pigment, it may be in the form of an ester or in combination with substances as yet unidentified.

The carbolfuchsin (acid-fast) staining reaction offers a better indication of the nature of the pigment, since this reaction is usually found in the tissues of animals fed tocopherol-deficient diets containing relatively high amounts of unsaturated fat. In vitro experiments of Endicott [19] likewise showed that the acid-fast reaction was found with unsaturated but not with saturated fats. Hass [20] has also suggested that this reaction may be due to polymerized peroxides of long chain fatty acids with several double bonds. The experiments reported here further verify the evidence that the acid-fast reaction of the tissues is correlated with the presence of unsaturated fats in the diet; for no adrenal pigment was found after a diet with a very low fat content or a diet with relatively saturated fat was fed. However, the pigment was found when diets containing unsaturated fat were fed or when fatty acids were added to the low fat diet. The qualitative and quantitative degrees of pigmentation were also related to the length of time the animals were fed such diets, for only small amounts of acid-fast pigment were found even in giant cells (fig. 14) after limited feeding of diets containing unsaturated fat.

Tocopherols are known to prevent deposition of acid-fast pigment in the tissues of rats fed tocopherol-deficient diets containing unsaturated fats. A sufficiently high dosage of tocopherols was likewise effective in preventing or limiting in the adrenal glands of mice the pigmentation due to the unsaturated fat contained in the diet. The inherent tocopherol content of the "crisco" diet, in addition to the relative sat-

16. Conn, H. J., and others: Biological Stains: A Handbook on the Nature and Uses of the Dyes Employed in the Biological Laboratory, ed. 2, Geneva, N. Y., The Commission on Standardization of Biological Stains, 1929, p. 75.

17. Whitehead, R.: J. Path. & Bact. **39**:443, 1934.

18. Everett, J. W.: Am. J. Anat. **77**:293, 1945.

19. Endicott, K. M.: Arch. Path. **37**:49, 1944.

20. Hass, G. M.: Arch. Path. **26**:956, 1183 and 1196, 1938; **27**:15, 1939; **28**:177, 1939.

uration of its fat component, also accounted for the absence of adrenal pigmentation in mice fed this diet with or without supplements of unsaturated fatty acids from cod liver oil.

A possible antagonism between tocopherols and estrogen was suggested by the clinical studies of Shute.[21] Such an antagonism would help to account for the presence of brown degeneration with prolonged estrogen treatment and the absence or inhibition of it with tocopherol therapy. However, when a preliminary test of this possibility was carried out with 8 spayed mice fed the stock diet and 2 mg. of tocopherols daily, all had vaginal cornification after being given a minimal dose of estradiol benzoate (0.20 micrograms) by subcutaneous injection, which would indicate that in the mouse such antagonism does not exist. This is in keeping with experiments by Halvorsen,[22] who also showed that tocopherols offered no counteraction to estrogen and progesterone treatment in the rabbit. Injected estrogen must, therefore, have altered the fat metabolism of the mice fed certain diets (stock, low fat and "crisco") to produce this pigmentation, for little or no pigment was found in the adrenal glands of animals fed these diets alone.

The type of cells that contain this pigment in the adrenal gland is not settled. Lacassagne and Reynaud [3a] suggested that the pigment found in the adrenal glands of mice after estrogen treatment was located in the reticuloendothelial cells. However, we observed this pigment in cortical parenchymal cells, also, which is in agreement with the reported capacity of such cells to phagocytose trypan blue.[23] Normally, the cortical cells considered to be the oldest (the inner reticular cells) usually are the first to contain this pigment. However, in advanced stages of brown degeneration, all areas of the cortex may be involved (fig. 4). The X zone cells appear to have the same ability to acquire this pigment as the cells of the outer cortical zones; for the adrenal glands of young whose mothers were fed the high lard diet during the last quarter of gestation and lactation contained pigment at 10 days of age, when most of the cortex consists of X-zone-like cells (fig. 8); moreover, before the X zone had degenerated normally or when it had been maintained by injections of estrogen (Vicari [24]), pigment was found within this zone (figs. 10 and 12). The X zone degenerated by vacuolation, as reported by others [25]; however, none of these degenerating cells con-

21. Shute, E.: Am. J. Obst. & Gynec. **44**:271, 1942; Urol. & Cutan. Rev. **47**:239, 1943.

22. Halvorsen, K.: Acta path. et microbiol. Scandinav. **21**:510, 1944.

23. Hett, J.: Ztsch. f. mikr. anat. Forsch. **7**:403, 1926. Cappell, D. F.: J. Path. & Bact. **32**:629, 1929.

24. Vicari, E. M.: Anat. Rec. **86**:523, 1943.

25. Howard-Miller, E.: Am. J. Anat. **40**:251, 1927. Deanesly, R.: Proc. Roy. Soc., London, s.B **103**:523, 1928. Whitehead, R.: J. Anat. **67**:387, 1933.

tained the pigment of brown degeneration (fig. 11). Pigmented cells were not found within the medulla as long as the X zone was present, but after the degeneration of the latter, pigmented individual or giant cells were found among the medullary cells. Once this pigment was formed in adrenal cells, they appeared unable to eliminate the pigment. It was also markedly resistant to postmortem autolysis.

From its staining reactions and its fluorescence under ultraviolet rays, the pigment of brown degeneration of the adrenal gland of the mouse appears to be similar to the ceroid of the dietary cirrhosis of the rat [26] or that found in the testes of mice treated with estrogens,[27] or the luteolipin of the corpus luteum of the rhesus monkey,[28] as well as the pigment found in the tissues of rats fed vitamin E–deficient diets with high contents of unsaturated fat.

SUMMARY

Brown degeneration was found in the adrenal glands earlier and in larger amounts after feeding diets with a high content of unsaturated fat to a strain of Swiss albino mice which has little of this pigment in the adrenal glands when fed a commercial stock diet with a low content of unsaturated fat, or no pigment when fed diets with very low fat or a relatively saturated fat content.

This pigment may be found in all zones of the adrenal cortex (including the X zone) as well as within the medulla in advanced stages of brown degeneration. No pigmented cells were found within the medulla if the X zone was present, but such cells were found in the medulla after the X zone had degenerated.

Injected estrogen appeared to increase slightly the formation of this pigment, whereas sufficient tocopherols (vitamin E) given in oral dosage or incorporated in the diet prevented or limited the formation of this pigment, provided the amount of unsaturated fatty acids in the diet was not excessive.

This pigment gave the Schultz cholesterol reaction in both frozen and paraffin sections. Cholesterol added to the diets in amounts to produce marked deposition of fat in the liver had little or no effect on the pigmentation of the adrenal cells.

From its staining reactions, this pigment appears to be similar to that found in the tissues of rats fed tocopherol-deficient diets with unsaturated fat content, the ceroid of the dietary cirrhosis of rats or that found in the testes of mice after prolonged estrogen treatment, and the luteolipin in the corpus luteum of the monkey.

26. Endicott, K. M., and Lillie, R. D.: Am. J. Path. **20**:149, 1944.
27. Frantz, M.: Anat. Rec. **97**:388, 1947.
28. Rossman, I.: Contrib. Embryol. **30**:97, 1942.

HETEROTOPIC BLOOD FORMATION IN EXPERIMENTAL CHOLESTEROL ARTERIOSCLEROSIS

RUDOLF ALTSCHUL, M.U.DR.

SASKATOON, SASKATCHEWAN, CANADA

IN A previous publication [1] it was asserted that experimental cholesterol arteriosclerosis is a good means by which to elicit various potential reactions of tissues. In the course of experiments of this kind [2] it was found that tissue changes and vascular changes occurred in the brain and the skeletal muscle of rodents, sites where such changes had not been seen before. In the following pages an additional alteration is reported, which, too, appeared during experimental cholesterol arteriosclerosis in rabbits and guinea pigs, namely, heterotopic blood formation. It was seen frequently in the cortex of the adrenal gland, a few times in the spleen but only once in the kidney and in the lymph node. So far I have been unable to detect it in the liver.

In studies of experimental arteriosclerosis, Chuma,[3] Versé [4] and Schönheimer [5] observed hemopoiesis in the spleen; Versé [4] and Anitschkow [6] observed it also in lymph nodes. In spite of great attention given to microscopic changes present in the adrenal gland, no hemopoiesis has as yet been observed in this organ.

Jordan in his general review [7] of extramedullary blood formation did not mention secondary sites other than the liver, the spleen and lymph nodes. Bloom [8] reported hemopoiesis occurring in the rabbit's adrenal gland after ligation of the renal artery and vein.

According to Brannan,[9] heterotopic bone marrow has been found in the adrenal gland; it was always accompanied by adipose tissue and

1. Altschul, R.:   Arch. Path. **42**:277, 1946.
2. Altschul, R.:   J. Neuropath. & Exper. Neurol. **5**:333, 1946; footnote 1.
3. Chuma, M.:   Virchows Arch. f. path. Anat. **242**:275, 1923.
.4. Versé, M.:   Verhandl. d. deutsch. path. Gesellsch. **20**:67, 1925.
5. Schönheimer, R.:   Virchows Arch. f. path. Anat. **249**:1, 1924.
6. Anitschkow, N., cited by Schönheimer.[5]
7. Jordan, H. E.:   Physiol. Rev. **22**:375, 1942.
8. Bloom, W., in Maximow, A. A., and Bloom, W.:   A Textbook of Histology, Philadelphia, W. B. Saunders Company, 1942, p. 115.
9. Brannan, D.:   Bull. Johns Hopkins Hosp. **41**:104, 1927.

was regarded as an embryonic defect. In anemic persons in whom hetero-topic hemopoiesis has a compensatory function, no adipose tissue was present.

MATERIAL AND METHODS

Twenty-eight rabbits and 12 guinea pigs were used in cholesterol feeding experiments. These animals were fed milk, yolk powder and yolk cake; some rabbits received capsules with 0.3 Gm. of cholesterol. In the latter case, the animals were given milk and yolk, too, in order to facilitate the absorption of pure cholesterol. The animals died spontaneously or were killed when their death seemed imminent. The organs and tissues were fixed in formaldehyde solution or Susa fixative and embedded in paraffin. The sections were stained by various methods, but for the present investigation only the hematoxylin-eosin stain appears relevant.

RESULTS AND COMMENT

Blood formation was found in the adrenal cortex, one kidney and one lymph node of rabbits and in the adrenal cortex and the spleen of guinea pigs. It was seen in the adrenal cortex quite frequently, but only those cases (7 rabbits and 1 guinea pig) were considered in which megakaryocytes were present. For these animals the duration of the experiments varied from eighty-three to two hundred and thirty-four days. The developing blood cells were of the erythroblastic and myelo-blastic series and were found sometimes in clusters but more frequently in rows or ribbons in the adrenal blood sinuses. The microscopic appear-ance was much in favor of intravascular hemopoiesis, originating from the endothelium. The hemopoiesis occurred either in the zona fascicu-lata or the zona reticulata of the adrenal cortex. A noteworthy fact was that the frequent marked alterations of the adrenal cortex did not prevent formation of blood in this organ.

In one kidney of a rabbit, young blood cells and megakaryocytes were observed between cortex and medulla, forming small clusters and ribbons in enlarged capillaries, the latter being surrounded by numerous foam cells.

As mentioned before, the spleen and one lymph node were also found to be hemopoietic, but since observations of hemopoiesis occurring in these organs have been reported by other authors, and since the spleen, the liver and lymph nodes often appear as secondary blood-forming organs, this fact will not be discussed further.

In the absence of megakaryocytes the formation of blood was not regarded as proved, even if the process was fairly obvious. It may well be assumed that in such cases continued search might finally have revealed some megakaryocytes, but as the number of "positive" cases was large enough to exclude an exceptional occurrence, unrelated to the experiment, the other cases were disregarded. It is quite probable that even other organs may be found ultimately to be the sites of heterotopic formation of blood now that attention has been called to this reaction.

The following questions arise:

1. Is the blood formation due to blood deficiencies, and has it nothing to do directly with the cholesterol feeding? Since the bone marrow suffers greatly in the course of experimental cholesterol feeding, the need for vicarious hemopoiesis appears quite plausible.

2. Is the heterotopic blood formation an embolic process?

3. Why is the adrenal cortex the site of blood formation rather than the liver and the spleen, which, by their embryonic development, are predestined to such an auxiliary function, as is seen in human pathologic conditions?

4. Is the heterotopic blood formation entirely a pathologic process, without a physiologic function?

To these questions, answers may be rendered as follows:

1. I was unable to find in the literature reports regarding leukopenias caused directly or indirectly by cholesterol feeding. Ignatowsky[10] observed, in his experiments with rabbits, a decrease in the number of erythrocytes and in the hemoglobin content. Okey and Greaves[11] observed anemia in guinea pigs which were on a cholesterol diet. Dubach and Hill[12] found macrocytic anemia. Vice versa, Kon,[13] and also Weinhouse and Hirsch,[14] found no changes in the blood of rabbits on a cholesterol diet, and Schwarz and Lichtenberg[15] reported a normal hemoglobin value. In my experience, which, however, does not extend to an examination of all animals used, blood counts and hemoglobin were normal. It also seems that the heterotopic hemopoiesis is too slight to compensate and hide any possible myelogenic anemia or leukopenia.

2. Heterotopic blood formation occurred in organs other than the adrenal cortex only in rare instances, so that an embolic displacement appears improbable. Moreover, the sinuses of the adrenal gland are wide enough to allow free passage of such cells and therefore would not retain them in clusters and ribbons.

3. The liver and the spleen are gravely affected in cholesterol arteriosclerosis. Much of the parenchyma of the liver is damaged, and most of the Kupffer cells are transformed into foam cells. In rabbits and, to a lesser degree by far, in guinea pigs the lymphatic tissue of the spleen is "outcrowded" by the immense number of foam cells and can well be called atrophic. Most of the elements of the reticuloendothelial system

10. Ignatowsky, A.: Virchows Arch. f. path. Anat. **198**:248, 1909.
11. Okey, R., and Greaves, V. D.: J. Biol. Chem. **129**:111, 1939.
12. Dubach, R., and Hill, R. M.: J. Biol. Chem. **165**:521, 1946.
13. Kon, cited by Chuma.[3]
14. Weinhouse, S., and Hirsch, E. F.: Arch. Path. **30**:856, 1940.
15. Schwarz, H., and Lichtenberg, H. H.: J. Biol. Chem. **121**:315, 1937.

are transformed into foam cells. Thus, in these two organs auxiliary blood formation may have been prevented by the pathologic process. However, one of the first organs to suffer in the course of cholesterol arteriosclerosis is the adrenal cortex. The cells of the parenchyma become enlarged as a result of an increase of their lipid content, and cholesterol crystals form in and between the cells. Moreover, necrotic areas with or without reparative inflammatory processes are seen. It seems, however, that only the parenchymal cells are grossly damaged; the lining cells of the sinuses show no distinct morphologic alterations. Thus heterotopic formation of blood from these cells may be possible. But it should be mentioned that Schönheimer [5] expressed the belief that in the course of cholesterol feeding the endothelial cells of the adrenal sinuses absorb fat.

4. One can hardly invoke in my cases an anemia with the necessity for an auxiliary formation of blood, especially since red and white cells are being formed heterotopically. It also appears that this formation of blood is not an embolic process. Moreover, it seems improbable that the damaged cortex would be chosen as the site for vicarious hemopoiesis (though this possibility should not be excluded). Therefore, one has to consider the possibility that the extramedullary blood formation observed in the adrenal gland and the kidney may be a pathologic process per se, and it might be classified as a regression of endothelium to an ontogenetically younger stage at which blood formation occurs. In this connection I wish to point out that a few years ago [16] I tried to demonstrate in the presence of human arteriosclerosis that endothelial cells had regressed to a younger, mesenchyma-like cell type. Thus, hemopoiesis occurring in the adrenal cortex and in the kidney in the course of cholesterol arteriosclerosis may well represent another incident of a regression of endothelial cells, for which one would have as the only factor suggesting an explanation the toxicity of excess cholesterol or its indirect action via damage of the liver.

It is noteworthy that other observers have not described hemopoiesis as occurring in the adrenal cortex during cholesterol feeding. As mentioned in the introduction, I was able to elicit changes in vessels of the brain and in skeletal muscle, although other experimenters have failed to do this, and I expressed the view that this discrepancy may be explained by the fact that I used heated (and thus probably oxidized) cholesterol. This same explanation may be applied to the present problem of heterotopic hemopoiesis. That is to say, the heterotopic formation of blood may be ascribed to the fact that in my experiments but not in those of the previous experimenters heat was used in baking the yolk cake, which increased the toxicity of the cholesterol. The regression or dedifferentiation of endothelial cells noted in both human and experi-

---

16. Altschul, R.: Arch. Path. **38**:305, 1944.

mental arteriosclerosis should not be considered as a proof that these processes are identical but only as an indication that under natural and experimental conditions the endothelium may react similarly.

SUMMARY

Extramedullary formation of blood occurs in association with experimental cholesterol arteriosclerosis. The question whether it constitutes an auxiliary function for aiding the damaged bone marrow is discussed. In the absence of anemia and leukopenia and because an embolic displacement can be excluded, the possibility is envisaged that the heterotopic hemopoiesis might be a pathologic process per se. In this case a regression of endothelium to the hemoblastic stage would be similar to the dedifferentiation of endothelial cells in human arteriosclerosis.

TOXICITY OF "STILBAMIDINE"
A Study of the Effects of Chronic Intoxication

LLOYD D. SEAGER, M.D.

AND

GINA CASTELNUOVO, Ph.D.

PHILADELPHIA

THE DIAMIDINES developed by York and others [1] have found many therapeutic applications.[2] They have been tried against trypanosomiasis, malaria, leishmaniasis and babesiasis, and as bacterial antiseptics. Their toxic action on tumor cells in vitro has been studied, and lately their action on multiple myeloma.

The pharmacologic and toxicologic studies of these agents have been incomplete. Moreover, some of the earlier studies have been complicated by the fact that infected animals were used.

Lourie and York [1] found that the maximum dose of "stilbamidine" (4,4-diamidinostilbene) tolerated is 50 mg. per kilogram by intraperitoneal injection in mice and 20 mg. per kilogram by intravenous injection in rabbits.

Similar results have been obtained by other investigators for "stilbamidine," "propamidine" (4,4'-[trimethylenedioxy]di-benzamidine) and "pentamidine" (4,4'-[pentamethylenedioxy]di-benzamidine).[3] Studies of acute and chronic effects have been conducted by several investigators.[4] The results have been variable but have indicated that the chief effects of the diamidines were on the liver and the kidneys.

---

1. Lourie, E. M., and York, W.: Ann. Trop. Med. **33**:289, 1939.

2. Fulton, J. D.: Ann. Trop. Med. **34**:53, 1940. Kirk, R., and Sati, M. H.: ibid. **34**:83, 1940. Adams, A. R. D., and York, W.: ibid. **34**:173, 1940. Kirk, R., and McDonald, D. R.: ibid. **34**:131, 1940. Daubney, R., and Hudson, J. R.: ibid. **35**:187, 1941. Snapper, I.: J. A. M. A. **133**:157, 1947; J. Mt. Sinai Hosp. **14**:119, 1946.

3. (a) Way, E. L., and Chan, L. K.: J. Pharmacol. & Exper. Therap. **81**:278, 1944. (b) Wien, R.; Freeman, W., and Schotcher, N. M.: Ann. Trop. Med. **37**:19, 1943. (c) Seager, L. D., and Rigdon, R. H.: Personal communication to the authors. (d) Broom, W. A.: J. Pharmacol. & Exper. Therap. **57**:81, 1936.

4. (a) Bischoff, F.; Sayhn, M., and Long, L.: J. Biol. Chem. **81**:325, 1929. (b) Devine, J.: Ann. Trop. Med. **32**:163, 1938; (c) **34**:67, 1940. (d) Daubney,

Our own experiments have been directed primarily to the study of the chronic toxic effect of "stilbamidine" as produced in rabbits and mice. "Stilbamidine" was administered to rabbits subcutaneously, while to mice it was given subcutaneously or by stomach tube.

MATERIAL AND METHODS

Rabbits and mice to which "stilbamidine" was given were killed at different periods after the administration of the drug. The following organs were fixed and studied histologically: heart, lung, liver, intestine, spleen, pancreas, kidney gonads and adrenal glands. No study was made of the nervous system.

The fixatives used were solution of formaldehyde U.S.P., Bouin's and Zenker's solution. Sections were stained with hematoxylin and eosin solution or with Pollack's trichrome stain. Sudan III was used for the study of fat in tissues and Best's carmine for the determination of the glycogen. We used 160 rabbits and 100 mice.

EXPERIMENTS ON RABBITS

The effects of doses of "stilbamidine" varying from 10 mg. up to 100 mg. per kilogram were studied; the dose was given in a single injection or was distributed in several injections. It was found that a single dose of "stilbamidine" of 75 mg. or more per kilogram kills a rabbit in the space of one or two days. An injection of 100 mg. per kilogram is generally lethal in the course of a few hours or a maximum of one or two days. If the amount of 100 mg. per kilogram is given not in one dose but in ten daily doses, the animal generally survives until a dose of 70 or 80 mg. per kilogram is reached. If a dose very close to the lethal dose (e. g., 50 or 60 mg. per kilogram) is given, the animal may not die in twenty-four or forty-eight hours. Generally speaking, it lives for one week. This delayed lethal effect has been investigated. During the course of treatment the rabbits lost weight, had some diarrhea, became weak and generally showed great congestion of the iris. Histologic sections of the iris showed large hemorrhages.

In order to determine the possible cause of the delay of the toxic effect in rabbits, several experiments were made. Since it is known that "stilbamidine" produced a fall in blood pressure, a suspension of epinephrine in oil, 0.02 to 0.013 mg. per kilogram, was given simultaneously with the "stilbamidine" and the treatment continued daily until the animal died. Epinephrine helped relieve the signs of toxicity in the period immediately following the injection but did not prevent the delayed effect of the drug. Experiments with ephedrine, 3 mg. per kilogram, gave the same result.

In order to counteract the loss of weight and of appetite, a solution of "amigen" and a solution of "amigen" and dextrose was given throughout some of the experiments, but no favorable results were observed.

Blood sugar and blood urea nitrogen were determined, and the van den Bergh test was made, in a group of 21 rabbits. Six were used as controls. To these a solution of sodium chloride was given once a week. The other 16 rabbits received

R., and Hudson, J. R.: ibid. **35**:187, 1941. (*e*) Kirk, R., and Henry, A. J.: ibid. **38**:99, 1944. (*f*) Allen, J. W.; Burgess, F., and Cameron, C. R.: J. Path. & Bact. **56**:217, 1944. (*g*) Oasther, E. G., and Fidler, H. K.: Tr. Roy. Soc. Trop. Med. & Hyg. **39**:533, 1946. (*h*) Cameron, G. R., and Oakley, C. L.: J. Path. & Bact. **38**:17, 1934. Wien and others.[3b]

"stilbamidine," 20 mg. per kilogram, once a week. Blood sugar and urea nitrogen were determined once a week, on the day preceding the injection of "stilbamidine." The qualitative van den Bergh test was made near the end of the experiment. Blood sugar was determined according to the Folin-Wu method; urea nitrogen, by Keller's [5] micromethod.

The blood sugar showed a variable response. Often there was preliminary hypoglycemia, occurring in three to four weeks; this was often followed by hyperglycemia, and occasionally the hypoglycemia returned. Tables 1 and 2 show the variations of the blood sugar level as determined for the control rabbits and for the rabbits which received "stilbamidine."

TABLE 1.—*Blood Sugar Levels of Controls*

Rabbit	Mg. of Sugar per 100 Cc. of Blood Before Injection	Mg. of Sugar per 100 Cc. of Blood on Given Day After Injection of Saline Solution							
		7	21	28	35	42	49	56	63
170	98	85	115	95	105	102	115	120	...
171	105	97	100	100	100	98	115	120	...
172	95	95	96	99	109	106	107	107	...
173	95	97	95	98	107	106	112	105	...
181	117	116	102	126	104	...	...	...	...
183	112	127	106	132	122	120	...	...	...

TABLE 2.—*Blood Sugar Levels of Rabbits Treated with "Stilbamidine"*

Rabbit	Mg. of Sugar per 100 Cc. of Blood Before Injection	Mg. of Sugar per 100 Cc. of Blood on Given Day After Injection of "Stilbamidine"							
		7	21	28	35	42	49	56	63
174	92	92	112	108	Expired				
175	98	109	100	95	170	105	130	115	145
176	94	94	90	60	200	195	120	115	158
177	90	98	103	65	140	102	68	120	157
178	94	100	82	52	124	98	110	175?	140
179	98	109	117	85	120	138	145	154	152
184	128	121	111	124	103	Killed			
185	113	129	104	135	169	Killed			
186	?	...	...	...	134	102	66	121	140
187	117	120	105	149	Died				
189	?	120	102	?	Died				
190	?	...	...	...	142	144	139	136	...
191	120	...	...	...	74	202	116	204	95

The qualitative van den Bergh test was made on serums of both the controls and the experimental animals. While the control serums always gave negative reactions, the experimental serums always gave positive reactions after the fourth injection.

The blood urea nitrogen was determined for all these animals, but the fluctuations found both for the controls and for the experimental animals were so great that we cannot draw any conclusions.

Blood counts, including differential counts, were made for a few rabbits each of which received an injection of "stilbamidine" once a week for eight weeks. The red and white cell counts did not change significantly during the entire course of the experiment. The differential counts generally showed a shift to the left toward the end of the experiment and especially in the period immediately following the

5. Keller, A. G.: J. Lab. & Clin. Med. **17**:1146, 1931-1932.

injection of "stilbamidine." Stab cells and juvenile cells and occasionally a few myelocytes were observed. A few nucleated red cells, cells with vacuolated cytoplasma and microcytes were occasionally observed. All these probably reflected the toxicity of the drug.

Pathologic Observations.—Of all the organs examined, only the liver and the kidneys were found to be affected by the drug, and therefore our description is limited to these two organs.

(*a*) Liver: Grossly, the livers of many of the rabbits which received a large amount of "stilbamidine" presented a mottled appearance. Histologically, those of rabbits which had 10 mg. per kilogram of the drug showed some dilatation of the blood sinusoids and of the central vein; the hepatic cells looked normal, and there was no fatty change. Rabbits which received 25 mg. per kilogram were killed after six months, and their livers were found to be perfectly normal. The same observations were made in regard to rabbits receiving 40 mg. per kilogram given in four doses of 10 mg. per kilogram each. The livers of rabbits which had 50 mg. per kilogram and were killed after three days showed dilatation of the sinusoids and some infiltration around the portal tract. The hepatic cells appeared normal.

The livers of rabbits which received 75 mg. per kilogram in doses of 25 mg. per kilogram and were killed at the end of the third week showed increased fibrous tissue and had large, dense masses of histiocytes and fibroblasts around the periphery of the hepatic lobules. Among these cells, several small, newly formed bile ducts were present. Also present among the infiltrating cells were some characteristic cells which contained fine brownish-pigmented granules. These pigmented granules have been found only in the livers of animals which received "stilbamidine." An iron-free pigment, "an *Abnutzung* (wear and tear) pigment," was described by Lubarsch (1899), according to Mitsuda,[5a] in necrotic liver cells.

The fibrous infiltration of the livers of some animals was so extended as to occupy a great part of the lobule itself. The hepatic cells which came to lie in contact with the fibrous infiltrating cells had a homogeneous and hyaline appearance; they took eosin heavily. Centrally, these cells were delimited by other cells, which were enlarged and had rather empty cytoplasm. Sometimes this appearance was due not to presence of fat or glycogen but to hydropic degeneration of the cells. Most of the cells in this region were binucleated. The centers of the lobules were usually formed by healthy cells, although foci of necrosis were sometimes noted among the central hepatic cells (fig. 1).

Rabbits which received 100 mg. per kilogram in the course of ten days had great cellular infiltration of the liver around the portal canal, predominantly of fibroblasts or histiocytes with a few lymphocytes. There was also proliferation of small bile ducts and fibrous tissue at the periphery of the lobules. The cells which were near the infiltrating cells showed the same characteristic changes as those in the preceding group.

In the livers of rabbits which had 75, 80 or 100 mg. per kilogram in only one injection and died within a few days, no infiltration or fibrosis was ever observed, and no necrosis was present. Only a slight fatty metamorphosis was observed. The only typical feature was dilatation of the blood vessels. There were only 2 rabbits which survived a dose of 100 mg. per kilogram given in one injection. They were killed and autopsies made at the end of seven months. In one of them an entire lobule of the liver was destroyed, and some hemorrhages were present. Around the periphery of the lobules there was a large infiltration of histiocytes, with some

---

5a. Mitsuda: Virchows Arch. f. path. Anat. **248**:91, 1924.
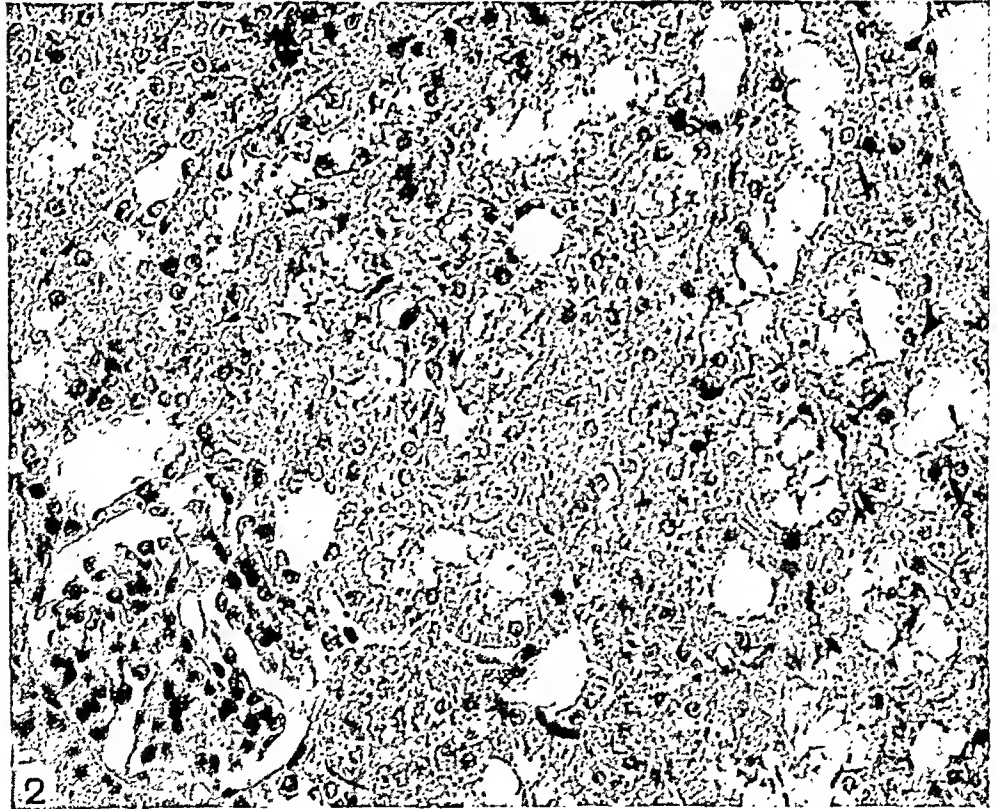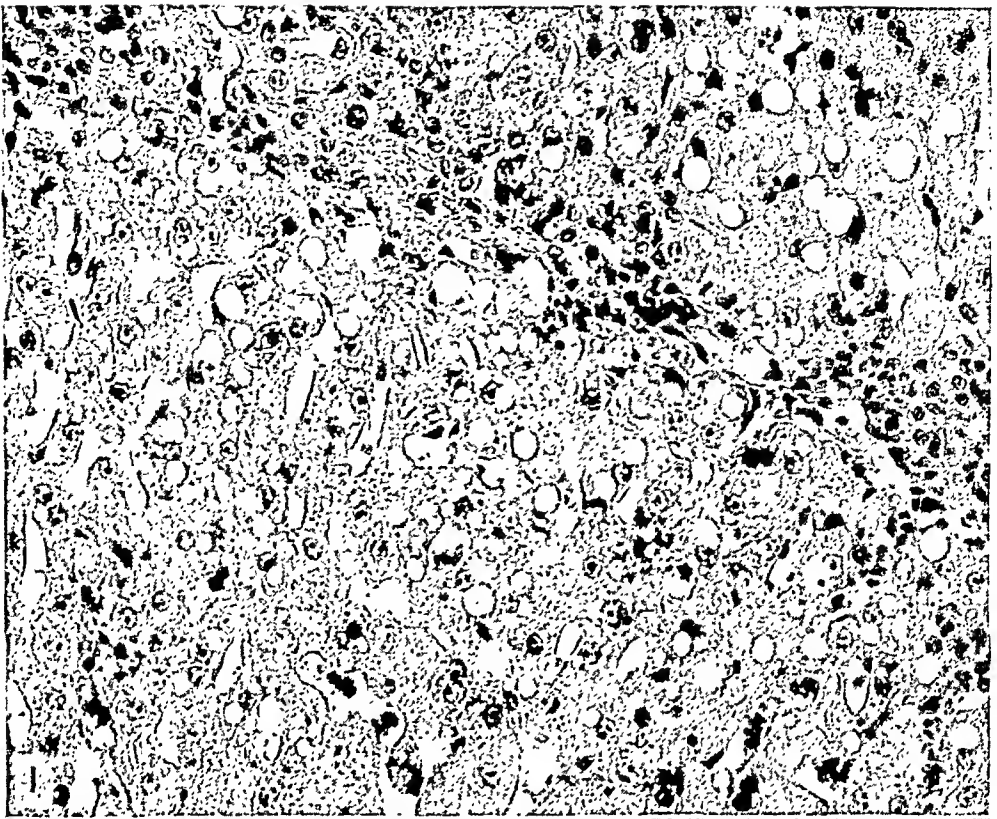
Fig. 1.—Photomicrograph of liver of rabbit 76; × 320. The rabbit received 60 mg. per kilogram of "stilbamidine" during three weeks.

Fig. 2.—Photomicrograph of kidney of rabbit 77; × 320. The rabbit received 60 mg. per kilogram of "stilbamidine" during three weeks.

fibrosis. The sinusoids were filled with blood. Glycogen was present in the liver cells. In the other animal, the liver was normal, and no fibrosis was present.

(*b*) Kidneys: Grossly, most of the kidneys looked normal, but some presented a pale cortex and a congested medulla. Histologically, the following features were observed: Rabbits which received doses of "stilbamidine" under 50 mg. per kilogram had some cloudy swelling in the convoluted tubules; in rabbits which received 50 mg. per kilogram in a five day period, the tubular cells were swollen and granular or reduced to fringes. Some glomeruli were swollen and enlarged.

In rabbits which received 60 mg. per kilogram during two weeks, cloudy swelling of convoluted tubules was extensive. The cells were either large and swollen and had very granular cytoplasm or were reduced to a narrow fringe. Staining with sudan III showed some fat in tubules, but only in a few cases was a large amount of fat observed.

With 75 mg. per kilogram the picture was about the same; cloudy swelling of various degrees was seen in the convoluted tubules and in the loops of Henle, while in the large collecting tubules the cells were very large and showed a rather vacuolated cytoplasm (fig. 2).

In rabbits which received 100 mg. per kilogram, either the tubular cells were so swollen that they entirely filled the lumens of the tubules or they were reduced to a narrow fringe. Glomeruli were often involved. When a glomerulus was involved, the tuft of capillaries was enlarged and reached the capsule in several places. In a few animals the glomeruli filled the entire subcapsular space. The glomeruli were often of different sizes in the same region.

In several animals many of the intertubular vessels of the medulla were intensely congested and enlarged. In some rabbits we observed the presence of arterioles with great hyperplasia of the intima and of the media. This was probably a manifestation of old arteriolosclerosis. Hyaline and sometimes granular casts were often noted, especially in the lower part of the medulla.

EXPERIMENTS ON MICE

The same organs which were examined in rabbits have been studied in mice. "Stilbamidine" was given by stomach tube or by subcutaneous injection. The mice were killed after one, two, three or more weeks of treatment. Doses of 10, 50 and 100 mg. per kilogram were given by mouth.

Mice given a subcutaneous injection of 50 mg. per kilogram daily died shortly after the doses totaled 550 mg. per kilogram. If death did not occur in a few hours after this total was reached, the mouse survived and never showed a delayed effect.

The changes observed after a short treatment of one or two weeks were dilatations of central and neighboring hepatic sinusoids and slight fatty metamorphosis, generally at the periphery of the lobules. After a long treatment, slight infiltration around the periphery of the lobules was observed, but it was never pronounced. Large cells whose cytoplasm was deeply stained with eosin were sometimes found. The size and the staining reaction of the nuclei of the hepatic cells were variable. In a few cases, isolated necrotic cells were noted (fig. 3).

The kidneys of the mice showed cloudy swelling as did those of the rabbits. The cells were swollen; many of the tubules were closed and often were filled with hyaline casts. In a few cases the glomeruli were swollen so as to occupy the entire subcapsular space (fig. 4). It is difficult to establish any relation between the dosage used and the characteristic appearance of the kidneys.
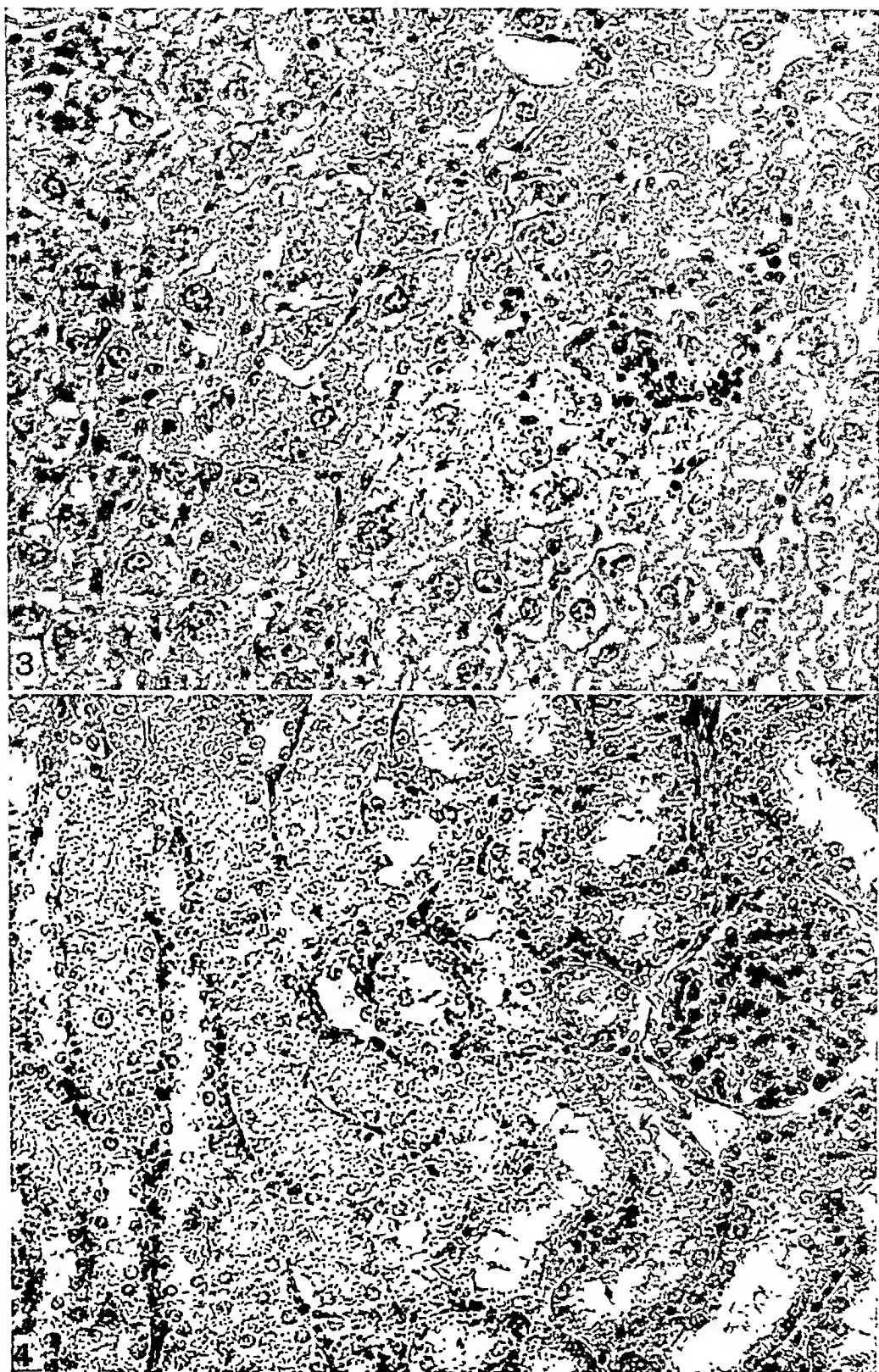
Fig. 3.—Photomicrograph of liver of mouse 70; × 320. The mouse received daily, by stomach tube, 10 mg. per kilogram of "stilbamidine" for a month.

Fig. 4.—Photomicrograph of kidney of mouse 70; × 320.

293

COMMENT

Our results compare in some respects with the results reported in the literature.

Large doses of aliphatic amidines, diamidines and diguanidines have been shown to affect the blood sugar and the blood urea nitrogen.[6] These changes have been correlated with hepatic damage.

Devine [4c] found that daily doses of 5 mg. per kilogram of "stilbamidine" given to rabbits by intravenous injection for six days did not produce any change in blood sugar or blood urea but that a single dose of 15 mg. per kilogram injected intravenously produced a sharp rise in blood sugar for a few hours, the concentration returning to normal in three days. A dose of 25 mg. per kilogram injected intravenously caused a short collapse, with the blood sugar rising to double the normal level.

In our experiments the blood sugar showed a variable response. There was often preliminary hypoglycemia, occurring in three to four weeks. This was followed by hyperglycemia, with an occasional return to a hypoglycemic level. The terminal blood sugar level was not always consistently elevated or depressed. In the experimental group the blood sugar varied from 52 to 202 mg., while in the controls it varied from 85 to 132 mg. per hundred cubic centimeters.

The average of the blood urea values was above the control level and fluctuated from 10.97 to 30.33 mg.

Other authors have studied the effects of the diaminides on various organs.

Gross and microscopic lesions produced by "propamidine" given to cattle have been described by Daubney and Hudson.[4d] They noted that marked fatty degeneration was produced in the liver.

Kirk and Henry [4e] found that the livers of animals receiving injections of "stilbamidine" presented fatty degeneration, accompanied by congestion. This fatty change occurred after fifteen minutes of an intravenous injection.

Wien, Freeman and Schotcher [3b] observed marked degeneration of the liver in healthy guinea pigs receiving 20 mg. per kilogram daily for five days. Doses of 4 and 8 mg. per kilogram given daily for five days produced no observable change in the liver, while in rabbits receiving 20 mg. per kilogram intravenously daily for six days they found moderate fatty degeneration of the liver.

Allen, Burgess nad Cameron,[4f] studying the toxic effect of "propamidine," observed hepatic and renal damage. They found that the production of necrosis of the liver was variable, sometimes affecting the peripheral zone of the lobules and sometimes the central zone.

---

6. Seager and Rigdon.[3c]   Bischoff, Sayhn and Long.[4a]   Devine.[4b]

In our experiments the peripheral zone of the lobules was generally affected. Large masses of infiltrating cells and proliferated small bile ducts were encountered at the periphery of the hepatic lobules when the rabbit received at least 75 mg. per kilogram over a period of three or more weeks. Fibrous infiltration is always more noticeable in animals which survive the treatment for at least a few weeks. This infiltration resembles the precirrhotic changes seen in various kinds of poisonings. None of our controls and none of the rabbits which received small doses showed such pronounced infiltration as rabbits which received large doses and were killed after several weeks. It has to be remembered that often adult rabbits show some infiltration at the periphery of the lobules and that many of the livers of healthy rabbits are diseased. It is therefore difficult to say whether, or to what extent, this infiltration is produced by the action of the drug. Scattered necrosis was sometimes observed and generally occurred at the periphery of the lobules.

Acute phosphorus poisoning is known to produce fatty degeneration and necrosis of liver cells in rabbits; in phosphorus poisoning of longer duration, some interstitial fibrosis and proliferation of bile ducts are obtained. These changes occurred in our animals, but since we never observed any real distortion of the regular pattern, we cannot speak of precirrhotic changes.

In poisoning with carbon tetrachloride, Cameron and Oakley [4h] found a definite increase in the number of some of the small bile ducts.

Kirk and Henry,[4e] reporting a study of the toxicity of "stilbamidine," said that extensive fatty degeneration was the most constant and typical change observed in the liver. In our experiments, extensive fatty degeneration was never found. The changes were limited to the periphery of the lobules.

Cells containing fine brownish-pigmented granules were generally noted at the periphery of the lobules among the infiltrating cells in the livers of rabbits which had been treated with "stilbamidine." An iron-free pigment was described by Lubarsch (1899) [5a] as found in necrotic liver cells.

Kirk and Henry [4e] also reported intense congestion of the kidneys with all degrees of tubular nephritis and areas of hemorrhage in the medulla as the consequence of administration of "stilbamidine." Our experiments on rabbits produced cloudy swelling of various degrees in the convoluted tubules and in the loops of Henle. The glomeruli were sometimes involved. In several animals, many of the intertubular vessels of the medulla were intensely congested and enlarged. Hyaline and sometimes granular casts have been found, especially in the lower part of the medulla.

It was observed that mice were more resistant to "stilbamidine" than rabbits, and that rabbits presented a delayed effect of the drug.

The livers of the rabbits showed heavier infiltration at the periphery of the lobules than the livers of the mice. Cloudy swelling of the convoluted tubules was found in the kidneys of mice, and hyaline casts were common.

Recently Oasther and Fidler [45] found cerebral lesions in 10 of 20 dogs treated with "stilbamidine," 1 mg. per kilogram being given daily during the first week, increasing to 1.5 mg. and to 2.0 mg. per kilogram during the second and the third week, respectively. In 2 animals, microscopic lesions of the brain stem, the cortex and the spinal cord were observed. In all except one of these dogs the liver was normal. Slight fatty degeneration was observed in the loops of Henle.

SUMMARY

"Stilbamidine" was administered subcutaneously to rabbits and subcutaneously or by stomach tube to mice to determine the morphologic and functional changes that might be caused by chronic intoxication with this drug. The changes, mainly degenerative, occurring in the organs most affected, namely, the liver and the kidneys, are described in detail.

HAIR GROWTH IN THE SKIN OF GUINEA PIGS PAINTED WITH 20-METHYLCHOLANTHRENE

MARTIN SILBERBERG, M.D.

AND

RUTH SILBERBERG, M.D.

ST. LOUIS

PROLONGED application of 20-methylcholanthrene produced in the skin of guinea pigs mild hyperplasia of the epidermis with increased mitotic proliferation of the basal cells. Although there were scattered hairless areas in the painted skin, growth of hair was rapid.[1] The present report deals with the histologic changes observed in hairs of the painted skin of these animals.

MATERIAL AND METHODS

The skin of 32 guinea pigs used in a former investigation of the effect of methylcholanthrene was studied.[1] In brief, the right ears and flanks were painted with a 3 per cent solution of 20-methylcholanthrene (dissolved in benzene) for periods of one-half, one, two, or three months. To the left ears and flanks of 16 of these animals benzene was applied; the left ears and flanks of the remaining 16 guinea pigs were not treated. Representative areas of the right side served as tests, and corresponding areas of the left side were used as benzene-treated or untreated controls.

Hair shafts and bulbs were counted in a standard area representing a low power field measuring 1.5 mm. in diameter, and averages were established on the basis of 100 fields counted in each animal. Mitoses were counted in the matrix of the hair (bulb) and the epithelium of the follicle. For this purpose, 1,000 sections of each, bulbs and shafts, were examined in ears and flanks of each guinea pig.

HISTOLOGIC EXAMINATION

In untreated skin, the hair was fairly uniformly spaced, and between the individual hairs the surface epithelium showed a more or less straight horizontal borderline toward the underlying cutis (fig. 1 *A*). The skin of the ear contained 3, and that of the flank 7, viable hairs in the standard area (table 1). Vegetating and dead hair *(Kolbenhaare[2])* were scarce. The ratio of bulbs to hairs was

1. Silberberg, M., and Silberberg, R.: Arch. Path. **43**:143, 1947.

2. Pincus, F.: Die Anatomie der Haut, in Jadassohn, J.: Handbuch der Haut- und Geschlechtskrankheiten, Berlin, Julius Springer, 1927, vol. 1, pt. 1, p. 1. Danforth, C. H.: Hair, in Malisoff, W. M.: Dictionary of Biochemistry, New York, Philosophical Library, Inc., 1943.
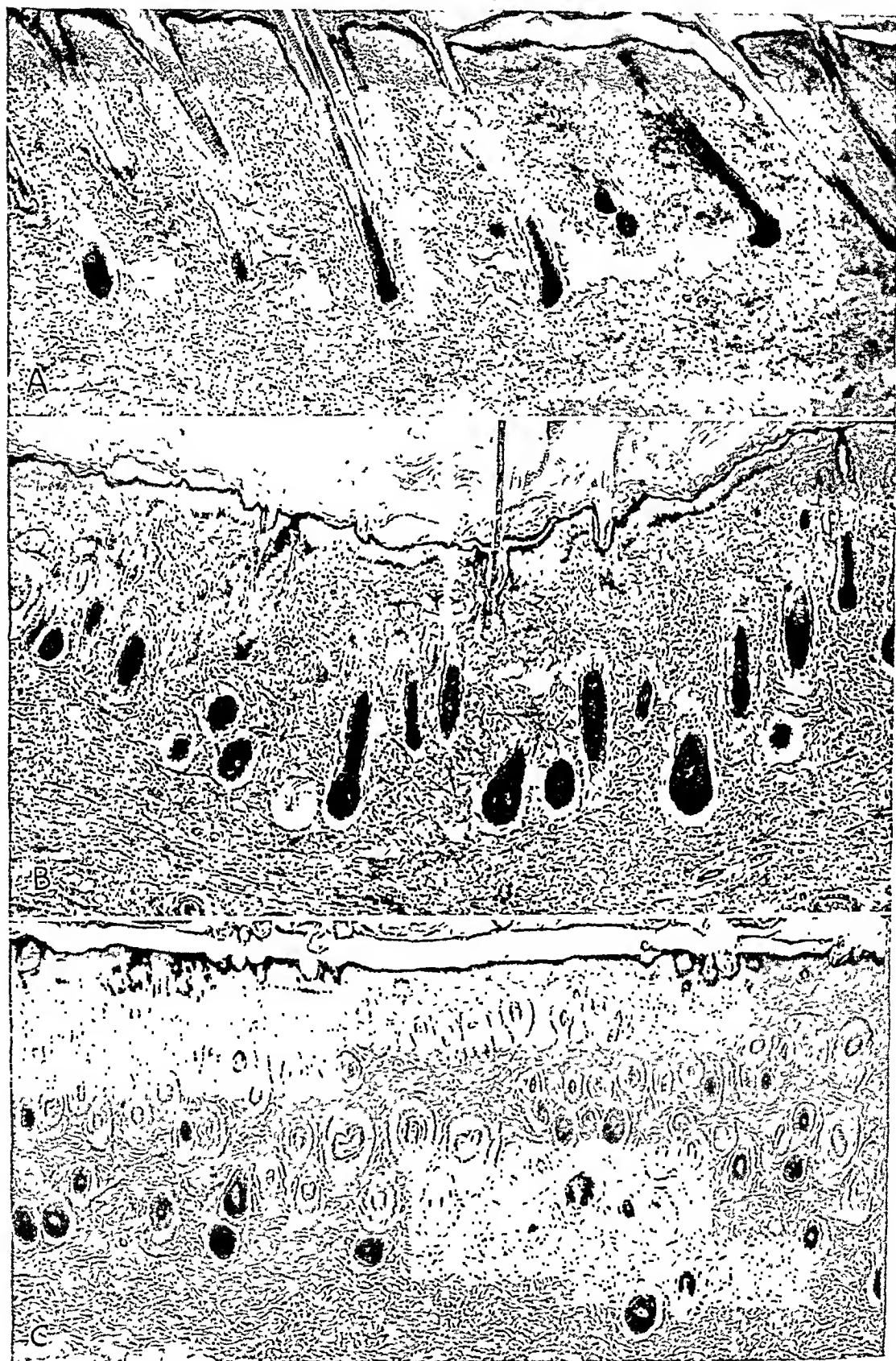
Figure 1
(See legend on opposite page)

10:11. Mitoses were more numerous in the matrix of the hair than in the epithelium of the follicle. The approximate numbers of mitoses per 2,000 cells (table 2) were: in the ear, 1.5 in the matrix and 0.5 in the epithelium of the follicle, compared with 3.5 in the surface epithelium; in the flank, 3.3 in the matrix and 1.1 in the epithelium of the follicle, compared with 2.5 in the surface epithelium.

After painting with benzene for one-half or one month, the hairs were more numerous and their distribution was more irregular than ordinarily (fig 1 *B*). They were definitely arranged in groups of three or four. The bulbs were enlarged, and some of them were situated somewhat more superficially than any in the nontreated control animal. Moreover, the slightly hyperplastic and hyper-

TABLE 1.—*Numbers of Viable Hairs and Epithelial Cords in a Lower Power Field*

Condition of Skin	Ear		Flank	
	Hairs	Cords	Hairs	Cords
Normal	3	.....	7	.....
Benzene painted				
½, 1 mo	4	.....	12-13	2-3
2, 3 mo	3-4	.....	12	.....
Methylcholanthrene painted				
½, 1 mo	4-5	2-3	12-14	8-9
2, 3 mo	4-5	1-2	13-14	3-4

TABLE 2.—*Number of Mitoses in Two Thousand Cells and in Multiples of the Normal (n) in the Matrix of the Hair, in the Epithelium of the Follicle and in the Surface Epithelium* *

Condition of Skin	Ear			Flank		
	Matrix	Follicle	Surface	Matrix	Follicle	Surface
Normal	1.5 (n)	0.5 (n)	3.5 (n)	3.3 (n)	1.1 (n)	2.5(n)
Benzene painted						
½, 1 mo	4.3 (3n)	1.8 (3½n)	5.2 (1½n)	6.0 (2n)	2.2 (2n)	3.8 (1½n)
2, 3 mo	4.2 (3n)	1.4 (3n)	5.3 (1½n)	2.9 (n)	1.0 (n)	3.5 (1½n)
Methylcholanthrene painted						
½, 1 mo	5.4 (3½n)	2.8 (5½n)	5.4 (1½n)	6.5 (2n)	2.8 (2½n)	5.2 (2n)
2, 3 mo	4.3 (3n)	1.5 (3n)	5.9 (1½n)	3.0 (n)	1.5 (1½n)	3.8 (1½n)

* The data for the surface epithelium are taken from the previous investigation and are added here for comparison.

trophic surface epithelium began to bulge into the thickened and congested dermis. The number of viable hairs of the standard area had increased to 4 in the ear and to 12 or 13 in the flank (table 1). Besides, there were in the latter 2 or 3 epithelial cords invaginating from the surface epithelium into the dermis. The

EXPLANATION OF FIGURE 1

A, normal skin of the flank (low magnification).

B, skin of the flank painted with benzene for one-half month (magnification as in *A*). The cutis is thickened; the hair follicles and bulbs are increased in number and size.

C, skin of the flank painted with methylcholanthrene for one-half month (magnification as in *A*). The cutis is thickened and contains a large number of vegetating or dead hairs and many hypertrophic bulbs.

Figure 2

(See legend on opposite page)

sebaceous glands were unmistakably enlarged. The ratio of bulbs to hairs had changed to 10:16. Many hairs were thicker than usual; many were well developed. Some representing vegetating or dead hair showed regressive changes, such as atrophy or swelling and hyalinization. The epithelial cells of the hair follicles were increased in number and size: In the ear there were 4.3 mitoses in 2,000 cells of the matrix (i.e., three times the normal figure) and 1.8 mitoses in 2,000 cells of the follicle (i.e., three and a half times the normal value). In the flank, 6 mitoses were found in 2,000 cells of the matrix and 2.2 mitoses in 2,000 cells of the follicle (i.e., twice the normal in both locations). After prolonged application of benzene the number of hairs (table 1) did not materially differ from that found at the earlier experimental stages. In the ear the mitoses remained at approximately the same level, whereas in the flank the mitoses in both the matrix and the epithelium of the follicle had fallen to normal (table 2).

Methylcholanthrene stimulated the growth of hair more than benzene did. After one-half or one month of painting, the hairs again were arranged in groups, but the grouping was less distinct than in the benzene-treated animals because of the further increase in the number and the thickness of the follicles (fig. 1 C) and the epithelial pegs; 4 or 5 viable hairs and 2 or 3 epithelial cords were counted in the ear, and 12 to 14 viable hairs and 8 or 9 epithelial cords in the flank (table 1). However, in contrast to conditions in normal or benzene-treated animals, there was a large number of vegetating and dead hairs (fig. 1 C) in the dermis. In some areas the bulbs were again situated closer to the surface than ordinarily (fig. 2 B). Not infrequently, 2 hairs were seen to emerge from the same follicular opening (fig. 2 A), but in many areas the hairs were broken off at the level of the skin surface (fig. 1 C). The cells·of the matrix and of the follicle had increased in number and size, and more so than had those in the guinea pigs painted with benzene. Regressive changes, in particular swelling and keratinization of the hair, were more accentuated and more widespread than after application of benzene. The hyperplastic and hypertrophic surface epithelium showed more numerous and deeper invaginations of the cutis as compared with that of the benzene-treated animals (fig. 2 C). Moreover, solid strands of epithelium budded out from the epithelium of follicles, usually from an area just above the origin of the sebaceous glands. These epithelial cords varied in length (fig. 3) and thickness, and many showed mitotic proliferation (fig. 3). The ratio of bulbs to hairs was 10:19. In the ear (table 2), there were 5.4 mitoses in 2,000 cells of the matrix (three and a half times the normal) and 2.8 mitoses in 2,000 cells of the follicular epithelium (five and a half times the normal). In the flank (table 2) the corresponding figures were 6.5 mitoses in 2,000 cells of the matrix (twice the normal) and 2.8 mitoses in 2,000 cells of the follicular epithelium (two and a half times the normal). After two or three months of application of methylcholanthrene there was no appreciable change in the number of hairs as

---

EXPLANATION OF FIGURE 2

A, skin of the flank painted with methylcholanthrene for one month (high medium magnification). The surface epithelium is hyperplastic, invaginating into the dermis at numerous points. Two hairs emerge from one follicular opening.

B, skin of the flank painted with methylcholanthrene for one-half month (low medium magnification). Note the small bulb close to the surface between the two sebaceous glands.

C, skin of the flank painted with methylcholanthrene for one-half month (high magnification). Several epithelial cords in the dermis show at their tips beginning formation of new bulbs and new dermal papillae.

Fig. 3.—Skin of the flank painted with methylcholanthrene for one-half month (high magnification). There is a long epithelial cord at the left side of the picture showing beginning bulb formation at the tip. An epithelial cord is budding out from the epithelium above the sebaceous gland. Note several mitoses in the epithelium.

compared with the numbers recorded at shorter stages, whereas both the growth processes and the regressive changes were less pronounced: In the ear 4 or 5 hairs and 1 or 2 epithelial cords, and in the flank 13 or 14 hairs and only 3 or 4 epithelial cords, were found in the standard area (table 1). Moreover, the mitotic counts (table 2) had declined: In the ear there were 4.3 mitoses in 2,000 cells of the matrix (three times the normal) and 1.5 mitoses in 2,000 cells of the epithelium of the follicle (three times the normal); in the flank the numbers of mitoses had dropped to normal or to one and a half times normal values, respectively.

COMMENT

In the skin of young guinea pigs, benzene and, more intensively, methylcholanthrene stimulated hair growth: The number of viable hairs was increased, particularly in the dermis of the flank; there were hyperplasia and hypertrophy of the epithelium of the follicles and of the matrices of the hairs. These findings are in agreement with the acceleration of hair growth observed grossly by Mottram[2] and by Butcher[4] under the influence of benzene or other irritants. Mitotic activity was stimulated in the follicles and in the matrices of the hair but less so in the flank than in the ear. Correspondingly, a return to normal was accomplished more readily in the former than in the latter. The stimulation of hair growth as indicated by the number of mitoses appearing in the matrices and the follicles did not progress, however, with prolonged application of methylcholanthrene. A peak was reached after one-half month. Thereafter the growth processes remained stationary or showed decline. Associated with this intensification of hair growth were regressive changes. These consisted of atrophy or of swelling and hyalinization of the hair, and many hairs were broken off near the surface. Again, the degenerative processes were more accentuated after application of methylcholanthrene than after that of benzene.

These results suggest a number of questions: (1) Is the increase in the number of hairs which was observed due to accelerated growth and replacement within the same follicle or (2) is there actual new formation of hair follicles? (3) Why does the epithelium of the hair follicles of the ear respond more vigorously to the growth stimuli than the surface epithelium of the ear? (4) What are the reasons for the difference in response of the epithelium of the ear and the flank?

Any increase in the number of viable hairs such as was observed under the influence of methylcholanthrene as well as under that of benzene cannot be discussed without touching on the moot question of whether or not new dermal papillae can be formed in postnatal life. Accelerated growth of the individual hair alone cannot account for the presence of a larger number of viable hairs. If one denies the new

3. Mottram, J. C.: Nature, London **155**:729, 1945.
4. Butcher, E. O.: Am. J. Physiol. **129**:553, 1940.

formation of papillae, one has to postulate accelerated formation of new hairs from the same papilla, possibly associated with delay in the shedding of the hairs. Under the influence of the growth stimuli the proliferation in the matrix may become so intensified that a new hair is formed immediately after the old one becomes detached from the papilla, without a period of rest such as normally occurs. The vegetating hair is still situated in the cutis as a new one or several new ones are being formed from below. This assumption seems to be corroborated by the findings of 2 hairs emerging from the same follicle (fig. 2*A*). Such an occurrence would constitute a relative delay of the shedding of old hair as compared with the rate of hair growth. But the shedding of the hair may also be absolutely retarded. The degenerating, hyalinized hairs might oppose greater resistance to the forces expelling them from the skin than the normal hair and thus stay inside the dermis longer than ordinarily. Degeneration of the hair is probably also the cause of the breaking-off of many of the hairs, which accounts for the hairlessness of some areas of the skin surface. This condition, thus, does not represent true epilation, since the corresponding areas of the dermis contain numerous viable hairs. It is also possible that the increased keratinization of the surface epithelium and the swelling and fibrillation of the connective tissue of the dermis caused by the irritants may render the extrusion of the old hair more difficult.

There are, however, indications that new hair may actually be formed from epithelium budding out from the follicles or from the surface epithelium of the skin. This mode of origin would necessitate the development of new bulbs including new cutaneous papillae such as occurs in embryonal or early postnatal life. Many epithelial pegs were found arising usually from the surface epithelium near the origin of sebaceous glands (fig. 2 *C* and 3). These epithelial cords varied in length, but quite a few showed at their dermal ends a small club-shaped thickening suggesting the new formation of a hair bulb. Some of these structures were situated close to the surface (fig. 2 *B*), and it seems plausible to assume that under the influence of epithelium growing out of the follicle new dermal papillae may develop. Even though new papillae may not develop in postnatal life under ordinary conditions, such new formation may occur under the influence of potent growth stimuli such as the polycyclic hydrocarbons. This conclusion should, however, be tested in further investigations.

A comparison of the growth processes of the epithelium of the matrix and the follicle with those of the surface epithelium shows differences in the degree of stimulation: Hair was more stimulated by both methylcholanthrene and benzene than was the epidermis. Benzene applied for a half or one month increased the number of mitoses three and a half times in the hair matrix of the ear but only one and a half

times in the surface epithelium. In the hair matrix of the flank the increase was twofold, compared with an epidermal increase of one and a half times the normal. Painting with methylcholanthrene for the same period multiplied the mitoses in the hair matrix of the ear three and a half times, compared with one and a half times in the epidermis; in the flank, on the other hand, the degree of mitotic proliferation was twice the normal in both hair matrix and surface epithelium. As seen from table 2, the stimulation of mitotic activity was even greater in the epithelium of the follicle (columns 4 and 7) than in that of the matrix of the hair (columns 3 and 6).

In mice painted with carcinogens, Mottram[3] observed similar differences in the response of surface and follicular epithelium and attributed them to a nonspecific factor, .namely, the better vascular supply of the dermal structures as compared with the surface epithelium. Additional factors may, however, also be involved. There may be a selectivity of the applied stimulus for the hair and the hair follicle. In mice painted with methylcholanthrene the carcinogen is retained in the sebaceous glands.[5] In the guinea pig conditions may be similar. However, whereas in the mouse the hair follicles are destroyed by the carcinogen, in the guinea pig they react with marked stimulation of hair growth. Whether this difference in response is related to the failure of tumors to develop in the skin of the guinea pig under the influence of cyclic hydrocarbons has to be analyzed further.

There was a marked difference in response between the hair of the flank and that of the ear. Under the influence of the stimuli applied, there was in the flank a one and a half to two and a half fold increase in the number of mitoses, and the number of hairs increased 80 to 100 per cent. In the ear, on the other hand, a three to five and a half fold rise of mitoses was associated with but a 30 to 50 per cent increase in the number of hairs. Thus conditions in the ear must be less favorable to the formation of hair as compared with those in the flank in spite of the relatively lesser increase in the number of mitoses in the latter under conditions of stimulation. Moreover, this discrepancy indicates that new formation of hair is not simply a function of the mitotic activity of the epithelium but that other prerequisites have to be fulfilled before new hair is formed. One of the factors involved is the cyclic character of hair growth.[6] In hair matrices and follicles, phases of growth alternate with periods of rest. The high and low points of the cycle occur at different times in different regions of the body surface, and different responses may be elicited, depending on whether the stimulus acts on resting or on actively growing hair. Moreover, the

5. Simpson, W. L., and Cramer, W.: Cancer Research **3**:515, 1946.

6. Trotter, M.: Arch. Dermat. & Syph. **7**:93, 1923.

apparent regional differences might be accounted for by differences in types of hair. Five types of hair, varying in length, thickness and shape, have been found in the guinea·pig.[7] It is possible that these types of hair differ not only in structure but also in growth rate. However, a true regional difference might be present, independent of the growth cycle and the type of hair and comparable to that found previously by Loeb[8] in the surface epithelium of the ear and of the flank in various animals: The epidermis of the ear is normally thicker than that of the flank, but the epithelium of the flank showed a more marked thickening under the influence of methylcholanthrene than that of the ear. In other words, there was a parallelism in the responses of surface epithelium and hair of each respective region: in the flank, marked thickening of the epidermis associated with marked increase in the number of hairs; in the ear, slight thickening of the epidermis accompanied by slight increase in the number of hairs.

Further investigations will have to answer more fully the questions arising from the present findings. Of special interest seems to be the inverse relationship between the growth stimulation of the hair and that of the surface epithelium. This condition exists not only in the guinea pig, which is resistant to the induction of cancer of the skin, but also in the mouse. In the latter, this inverse relation ends with tumors being formed in the epithelium after destruction of hair follicles.

SUMMARY

In young guinea pigs benzene and, still more intensely, methylcholanthrene stimulate hair growth: Mitotic proliferation and hypertrophy of the epithelium of the hair matrix and the hair follicle are increased, and there are indications that under the influence of the carcinogen new hair follicles and dermal papillae may be formed. This conclusion, however, should be tested in further investigations. Maximum growth is reached after approximately one-half month of treatment. Associated with the intensification of growth are regressive changes consisting of atrophy or swelling and keratinization of hair. The matrix of the hair and the epithelium of the follicle react to these experimental stimuli more actively than the epidermis. Hair growth is more stimulated in the flank than in the ear.

7. Dawson, H. L.: Am. J. Anat. 45:461, 1930.
8. Loeb, L., and Addison, W. H. F.: Arch. f. Entwcklngsmechn. d. Org. 37:635, 1913. Loeb, L., and Spain, K. C.: J. Exper. Med. 25:107, 1916; J. M. Research 41:247, 1919.

ULTRASPECTROPHOTOMETRIC STUDIES OF EXTRACTS OF NORMAL AND OF TUMOR TISSUE OF HUMAN ORIGIN

MONA SPIEGEL-ADOLF, M.D.

AND

MACHTELD E. SANO, M.D.

PHILADELPHIA

HISTOLOGIC properties in many instances have a chemical correlate traceable after the destruction of the cell structure. It seems highly probable that the great number of cells in malignant tumors, the frequent and atypical cell division and the hyperchromatism would become manifest in the extracts as an increase of the nucleic acids or of their cleavage products. Various authors (Claude and Rothen [1]; Thomas [2]; Stowell [3]) have even suggested that nucleic acids or their protein compounds may be an essential part of the active principle of tumor formation. When the properties of malignant growths are compared with similar properties of normal tissues or nonmalignant tumors, quantitative differences are to be expected (Davidson and Waymouth [4]; Schneider [5]), although qualitative ones may be possible. For the detection of nucleic acids, ultraspectrography has been used advantageously (Dhéré [6]; Spiegel-Adolf and Seibert [7]; Caspersson [8]; Spiegel-Adolf and Spiegel [9]). Our studies were therefore based on ultraspectrographic analyses of tissue extracts.

1. Claude, A., and Rothen, A.: J. Exper. Med. 71:619, 1940.

2. Thomas, P. T.: Nature, London 156:738, 1945.

3. Stowell, R. E.: Cancer Research 5:788, 1945.

4. Davidson, J. N., and Waymouth, C.: Biochem. J. 38:379, 1944. .

5. Schneider, L. C.: Cancer Research 5:717, 1945.

6. Dhéré, C.: Recherches spectrographiques sur l'absorption des rayons ultra-violets par les albuminoïdes, etc., Thesis, Fribourg, 1909.

7. Spiegel-Adolf, M., and Seibert, F. B.: J. Biol. Chem. 106:373, 1934.

8. Caspersson, T.: Skandinav. Arch. f. Physiol., 1936, vol. 73, supp. 8. (a) Caspersson, T., and Schultz, J.: Nature, London 143:602, 1939. (b) Caspersson, T., and Thorell, B.: Chromosoma 2:132, 1942.

9. Spiegel-Adolf, M.; Spiegel, E. A.; Ashkenaz, E. W., and Lee, A. .S.: J. Neuropath. & Exper. Neurol. 4:277, 1945.

MATERIAL AND METHODS

Tissues were available from 30 patients. With the exception of 4 specimens, the material was obtained at operations. According to the outcome of clinical and histologic examinations and tissue cultures, 14 specimens were from malignant tumors, 1 was a lymph node from a patient with Hodgkin's disease, 1 was a lymph node from a patient with lymphatic leukemia and 3 came from benign tumors. Seven inflammatory lymph nodes and a specimen showing inflammatory hyperplasia of a breast gland, as well as 5 samples of various normal tissues, served as controls. One specimen came from a patient whose condition was clinically questionable and who is still under observation.

For the preparation of tissue extracts, the following technic was uniformly used: Approximately 1 cc. of tissue was finely cut with iris scissors and suspended in 3 cc. of isotonic solution of sodium chloride (p_H 7.2 to 7.4). The suspension was heated for one-half hour at 65 C. to inhibit the enzyme action (Greenstein [10]). After the suspension had been centrifuged at 2,000 rotations per minute for ten minutes, the volumes of the tissue and the saline solution were measured in the graduated centrifuge tube to establish the ratio between tissue and fluid extract. Sedimentation was induced by centrifuging the suspended material at 9,000 rotations per minute for thirty minutes in an angle centrifuge. The entire procedure was done under sterile conditions, and when, as a final stage, the extract was cultured in broth and on blood agar plate it was shown to be sterile except in 2 cases of postmortem specimens. The extracts were kept in the ice box between the single stages of preparation and when not in use.

For the ultraspectrophotometric studies a DU Beckman quartz spectrophotometer was available. It was used with a 5 mm. quartz cell. As most of the tissue extracts were opalescent, dilutions were made with isotonic solution of sodium chloride. According to a preliminary test, some of the solutions did not comply with the law of Beer (i. e., the extinction coefficients were not independent of the degree of dilution). It seemed therefore preferable to compare the solutions as to optical density in the same quartz cell at equal degrees of dilution. The measurements were made in steps of 10 angstrom units between 3,100 and 2,200 angstrom units. In some preliminary tests the effectiveness of the working conditions was ascertained. The heated extracts maintained their optical absorption power on standing in the ice box, while the unheated showed a marked decrease. Centrifuging at less than 9,000 rotations per minute for thirty minutes was not enough in clearing up the extracts. Centrifuging at 9,000 rotations per minute could be repeated for another period of thirty minutes without altering the optical absorption power of the tissue extracts. In contradistinction, 16,000 rotations per minute produced a marked decrease in the spectrophotometric results.

The optical studies of our tissue extracts gave the following results: Normal leukocytes when treated like the cell suspensions to which they were quantitatively comparable (equal length of centrifuged column) showed a faint indication of selective absorption at 2,600 angstrom units, but the graph looks rather S shaped within a wavelength range between 2,800 and 2,500 angstrom units. Hemolyzed blood, on the other hand, showed selective absorption only at 2,750 angstrom units, an optical behavior characteristic of the proteins.

---

10. Greenstein, J. P.: The Biochemistry of Cancer, New York, Academic Press, Inc., 1947, p. 127.

Normal tissues of a high cell content show different spectrophoto-[1] metric behavior. Liver is characterized by selective absorption with a peak at 2,650 angstrom units, confirming the findings of Carter and Greenstein.[11] Endometrium (late secretory stage) from a case of fibro-myoma uteri showed similar results. The findings in the liver may be partly explained also by the role which, according to Barnes and Schön-heimer,[12] this organ plays in the synthesis of purines and pyrimidines. The selective absorption of the endometrium is not surprising in view of its amazing regenerative capacity [12a] and its sensitivity to hormone stimu-lation. Further studies will be necessary to elucidate this problem. Seven inflammatory lymph nodes gave uniformly selective absorption, with the peak in 6 cases at 2,500 angstrom units. An inflammatory tumor of the breast gave, on the other hand, only an S shaped optical absorp-tion graph. The results of the study of tumor extracts indicated likewise great uniformity. Of 10 carcinomas, 9 showed selective absorption, and only 1, a scirrhous carcinoma of the breast, showed an S shaped graph. Of the 9 specimens, 8 had a peak at 2,600 angstrom units, the height of which seemed in certain relation to the number of cells of the neoplastic tissue. This became clearly manifest in our 3 mammary tumors. Only 1 specimen, a medulloblastoma, was distinguished by two peaks, at 2,550 and 2,700 angstrom units. But it seems probable that the high lipid content of this preparation modified the properties of the tissue extract. Of 3 specimens of lymphosarcoma, 2 which were clinically and histologically similar gave nearly identical absorption graphs, with peaks at 2,600 angstrom units. The third, which gave an atypical his-tologic picture and which was diagnosed on the basis of the outcome of tissue culture, gave an atypical absorption graph likewise. A specimen from a patient with Hodgkin's disease gave findings similar to those noted with the 2 lymphosarcomas, while the absorption peak for tissue involved in chronic lymphatic leukemia was at 2,500 angstrom units as in the inflammatory nodes. Of the 3 benign tumors available, a fibromyoma and a hemangioma did not show selective absorption. One fibromyoma which was studied at nearly double the usual concentration showed a low peak at 2,550 angstrom units.

COMMENT AND CONCLUSIONS

As nucleic acids are normal constituents of tissues, it is obvious that differences in the characteristic absorption bands noticed with malig-nant tumors are primarily of a quantitative character. Our findings con-firm those of Davidson and Waymouth,[4] according to whom tumor

11. Carter, C. E., and Greenstein, J. P.: J. Nat. Cancer, Inst. **7**:51, 1946.
12. Barnes, F. W., Jr., and Schönheimer, R.: J. Biol. Chem. **151**:123, 1943.
12a. Brues, A. M.; Tracy, M. M., and Cohn, W. E.: Science **95**:558, 1942.

tissue has usually a higher nucleic acid content than its tissue of origin. No explanation can yet be offered for the facts that the peak of selective absorption is about 2,650 angstrom units for normal liver and endometrium, 2,600 units for carcinoma and 2,500 units for inflammatory lymph nodes. Tentatively, it could be suggested as Lavin, Loring and Stanley [13] assumed for tobacco mosaic virus, and one of us (Spiegel-Adolf [14]) for cerebrospinal fluids, that selective absorption at 2,650 angstrom units is due to the influence of proteins on nucleic acids. The peak
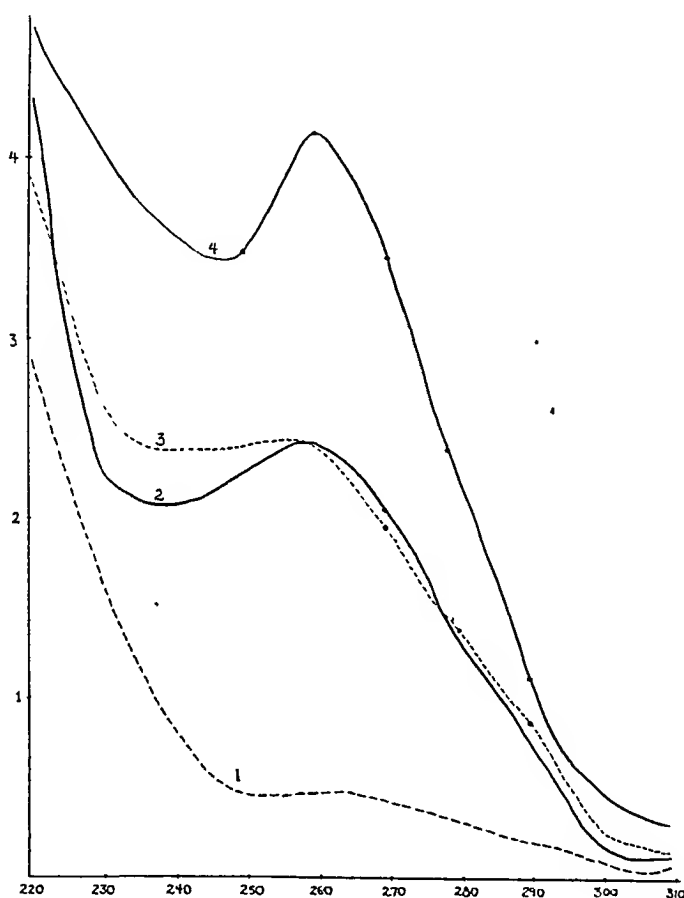
Fig. 1.—Optical absorption graphs of tissue extracts from various tumors of the breast (dilution of extracts, 1:4): *1*, scirrhous carcinoma; *2*, medullary carcinoma; *3*, inflammatory hyperplasia; *4*, medullary carcinoma, highly cellular type.

of 2,600 units for tumors could be explained by more extensive breakdown of nucleoproteins. In the presence of brain tumors cerebrospinal fluids may show similar selective absorption; this may be due, at least partly, not only to nucleoproteins or their derivatives but also to other

13. Lavin, G. J.; Loring, H. L., and Stanley, W. M.: J. Biol. Chem. **130**:259, 1939.

14. Spiegel-Adolf, M.; Wycis, H. T., and Spiegel, E. A.: J. Optic. Soc. America **35**:800, 1945; Federation Proc. **5**:156, 1946.

absorbing substances—for example, ascorbic acid (Spiegel-Adolf and Wycis [15]). The question why the absorption peak of inflammatory nodes is at 2,500 angstrom units will have to be made the subject of further studies. According to Vlès and Kagans,[16] the absorption peak of pure ribonucleic acid with p_H 4.7 is at 2,600 angstrom units. Shifts of this peak as far as 2,550 angstrom units have been observed by these authors between p_H 5 and p_H 10. Stimson and Reuter [17] were unable to observe a noticeable shift in the absorption peaks of either purified yeast or thymonucleic acids at various p_H values. It is questionable anyhow how far
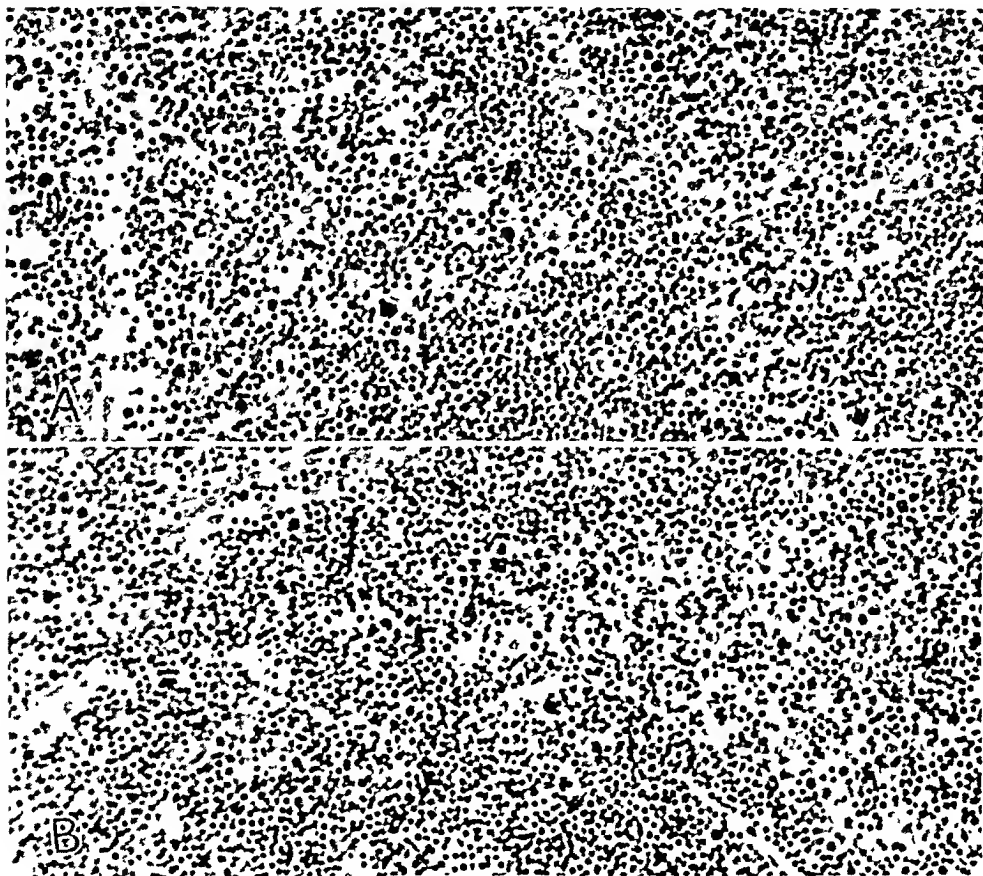
Fig. 2.—Histopathologic comparison of lymph nodes: *A*, lymphosarcoma; *B*, lymph node showing inflammatory hyperplasia. Hematoxylin and eosin stain; × 200.

changes in the hydrogen ion concentrations of inflammatory tissues become manifest in our tissue extracts. Nevertheless, a few practical conclusions may already be attempted on the basis of the present material. Our results allow us to differentiate between different kinds of

15. Spiegel-Adolf, M., and Wycis, H. T.: J. Phys. Chem. **50**:447, 1946.
16. Vlès, F., and Kagans, R.: Arch. de physique biol., 1943, vol. 16, supp. 110.
17. Stimson, M. M., and Reuter, M. A.: J. Am. Chem. Soc. **67**:847, 1945.

tumors of one organ, a finding which may be of prognostic importance (fig. 1). From the diagnostic point of view, the behavior of the lymph nodes under various pathologic conditions seems of interest. A count of cells alone does not explain the spectrophotometric behavior. Figure 2 shows the specimens equally rich in cells; one an inflammatory lymph node, the other a lymphosarcoma. Nevertheless, the peak of the extract of the first is at 2,500, the other at 2,600, angstrom units. Figure 3 visualizes those spectrographic differences, adding a specimen from a case of chronic lymphatic leukemia which shows optical absorption
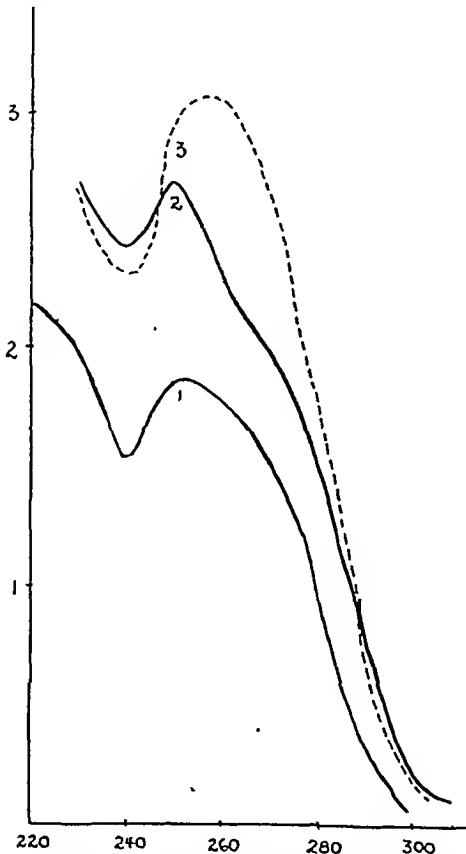
Fig. 3.—Optical absorption graphs of tissue extracts from pathologic lymph nodes (dilution of extracts, 1:9): *1*, chronic lymphatic leukemia; *2*, inflammatory hyperplasia; *3*, lymphosarcoma.

similar to that of inflammatory lymph nodes. It seems of interest in this respect that, besides the histologic likeness to the inflammatory lymph node, the antigenic properties of the leukemic lymphocytes and the normal ones cannot be distinguished.[18] Although no quanitative considerations are included, we wish to point to the fact that in figure 3 the highest peak is shown by lymphosarcoma. This may be expected from the nucleic acid content, while ascorbic acid, giving absorption in

18. Steinberg, B., and Martin, R. A.: Am. J. Path. **22:**652, 1946.

the same range, seems to be less concentrated in lymphosarcoma than in normal lymph nodes, according to Greenstein's [10] observations regarding rats. Finally, we wish to mention a case which illustrates the specificity of our findings. A small tumor metastasis could be separated from the rest of a lymph node. Tissue extracts and microscopic slides were made from each part separately. The spectrophotometric findings confirmed the histologic ones, the tumor part showing an absorption peak at 2,600 angstrom units and the inflammatory lymph node part having its maximum absorption of 2,500 units.

SUMMARY

Ultraspectrophotometric studies of extracts of normal and of tumor tissue of human origin were made with the following results: Tissues from carcinoma and lymphosarcoma gave practically uniformly selective absorption peaks at 2,600 angstrom units. Extracts from inflammatory lymph nodes had their maximum of selective absorption at 2,500 units, while normal liver and endometrium had their height of absorption at 2,650 units.

Case Reports

LEIOMYOMA OF THE FEMALE BREAST

JOHN M. CRAIG, M.D.
BOSTON

THE LITERATURE contains fairly numerous references to a small, smooth muscle tumor arising from the nipple or the areolar region of the breast. The cases have been collectively reviewed by Melnick,[1] Driak and Sternberg,[2] Schauder[3] and Lindfors.[4] This tumor is generally considered to arise from the smooth muscle of the papillae mammae and the lactiferous ducts. There is a smooth muscle tumor of the breast proper, unrelated to the nipple or the skin, which is rare; its origin is equally obscure. Stein[5] admitted only 5 cases of this type; he excluded cases of myosarcoma and myoepithelial tumor.

REPORT OF A CASE

M. S., a 40 year old Negro woman, married, who had been pregnant twice but had borne only one full term, living child, had a mass in the left breast. This mass was first noted ten years previously, shortly after the birth of her only child. It remained stationary in size and was painless until three months before she entered the hospital. During the latter period the patient noted twinges of pain in the involved region in the course of her menstrual periods. There was no fluctuation in the size of the mass, nor were there any other symptoms related to the breasts. The patient had received antisyphilitic therapy during her pregnancy and again for the last two years before her hospitalization. On physical examination, the breasts were heavy and pendulous, and the left breast was definitely enlarged. The nipple and the areola were normal. In the inferolateral quadrant of the left breast, 9 cm. from the nipple, there was a sharply demarcated, resilient mass the size of a baseball. It was attached neither to the skin nor to the underlying muscle. The remainder of the left breast and the right breast were normal to palpation. The axillary lymph nodes were not enlarged. The blood Hinton reaction for syphilis was negative.

At operation a 5 cm. linear incision was made over the center of the tumor. The tumor was encapsulated and was shelled out from the rest of the breast along an easily definable plane of cleavage. No further resection of the breast was considered necessary, and the wound was closed in layers.

The mass was found to measure 10 by 8 by 4 cm. and to weigh 162 Gm. The external surface was smooth except where its ellipsoid shape was distorted by variously sized rounded protuberances having diameters up to 3 cm. These rose

From the Department of Pathology, Peter Brent Brigham Hospital.

1. Melnick, P.: Arch. Path. **14**:794, 1932.
2. Driak, F., and Sternberg, H.: Deutsche Ztschr. f. Chir. **207**:353, 1928.
3. Schauder, H.: Deutsche Ztschr. f. Chir. **205**:58, 1927.
4. Lindfors, A. O.: Monatschr. f. Geburtsh. u. Gynäk. **11**:763, 1900.
5. Stein, R. J.: Arch. Path. **33**:72, 1942.

above the general surface of the mass for distances of 0.5 to 2 cm. The surface of the tumor was a uniform pinkish white. On palpation the mass consisted of many rounded firm nodules of varying sizes set in a softer intervening tissue. Hemisection of the tumor showed numbers of white nodules rising above the general plane of
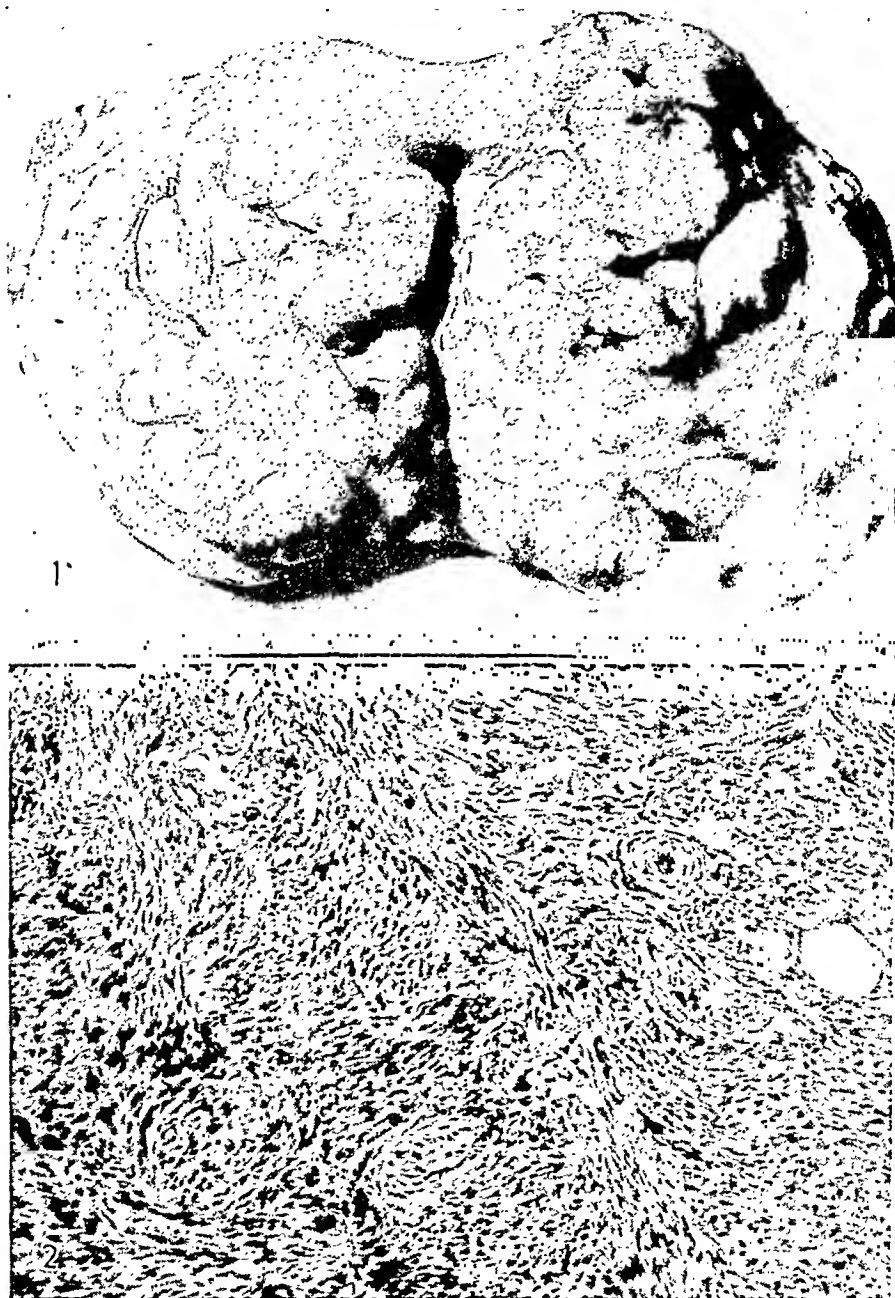
Fig. 1.—Cross section of the tumor showing the nodular character of the mass.

Fig. 2.—Microscopic section of the tumor showing the arrangement of the muscle bundles and their relation to the blood vessels. Eosin methylene–blue; × 106.5.

the cut surface for distances up to 0.5 cm. These nodules ranged from pinpoint-sized dots to irregularly rounded masses 3.0 cm. in diameter. The softer intervening stromal tissue was translucent, slightly rubbery in consistency and yellowish white.

Nine separate samples of the tumor were taken from distinctive portions of the specimen for microscopic examination. The sections were fixed in Zenker's solution to which acetic acid had been added to the concentration of 5 per cent and 4 per cent solution of formaldehyde and were stained with eosin–methylene blue, Mallory's aniline blue connective tissue stain and phosphotungstic acid–hematoxylin. The predominant feature of these sections was the presence of numerous variously sized groups of smooth muscle cells having a closely related and intimately mixed fibrous tissue stroma. The smooth muscle cells appeared in wavy continuous bundles, five to six cells in width, and as single cells almost isolated by the fibrous stroma cells. The smooth muscle cells were characterized by elongate regular nuclei with rounded ends and prominent small clumps of chromatin, and by longitudinal myoglial fibrils. These fibrils took a deep blue stain with phosphotungstic acid–hematoxylin and retained the acid fuchsin when stained with Mallory's aniline blue connective tissue stain. In comparison with these, the stromal cells had nuclei that were more vesicular, with much more variation in nuclear size and shape; some were almost round, while others were long and undulating. They were seen closely applied to collagen bundles. In some areas the tumor mass formed a capsule by compression of the surrounding tissue; yet in others the smooth muscle bundles faded off into a loose meshwork of edematous, acellular, fibrous interlobular breast tissue. In a few areas this interlobular tissue appeared to have become necrotic, the individual fibers breaking up and the nuclei disappearing. Normal mammary lobules were seen between the tumor masses, but in no case were they involved by the tumorous process; they were surrounded by a dense, hyalinized collagenous matrix. Among the smooth muscle bundles, the blood vessels were numerous, and the smooth muscle fibers could be seen curving about them. In some areas there was considerable hyperplasia of the fibrous layers of the smaller arterioles with distinct deposition of collagen between the cells; this was reminiscent of the hyperplasia of the adventitial layers of blood vessels seen in some meningiomas. In no case did the circular muscle fibers of the blood vessels take part in the tumor.

The reported cases of pure leiomyoma of the breast are gathered with the present one in the accompanying table. The 2 cases of smooth muscle tumor reported by Klob [6] and cited by Stein [5] are omitted from the table because the specimens are not adequately described. From a perusal of the table it is seen that leiomyoma of the breast occurs in women in the fifth and sixth decades. In all of the patients, however, the tumor had been present a long time before its removal; this fact emphasizes its slow rate of growth. In each instance the tumor was large and encapsulated, and in most cases, freely movable. In some instances it was of an almost uniform consistency, broken only by one or more cystic structures; in others it was made up of many nodules, variously sized. In the majority of the cases the tumor was quite vascular and the fibrous stroma prominent.

The origin of leiomyoma of the breast is still a matter of speculation. Melnick [1] suggested that it may arise (1) from a teratoid tumor

6. Klob, J. M.: Pathologische Anatomie der weiblichen Sexualorganen, Vienna, W. Braumüller, 1864, p. 492.

with an overgrowth of the myomatous elements, (2) from smooth muscle elements displaced from the nipple region, (3) from the myoepithelial layer of the ductal structure and (4) from the muscular portions of the blood vessels. He favored the latter explanation for its origin in the case which he described. The case described by Strong[7] lends support to this theory because of the intimate relation of the

Cases of Pure Leiomyoma

Author	Clinical Data	Size and Location	Gross Description	Microscopic Examination
Strong[7]	46 yr. old woman; duration of tumor 4 yr., with rapid growth last 2 yr.; discomfort 6 mo.	6 x 3 cm. oval mass; distinct from nipple; attached to underlying fascia	Encapsulated, firm, fibrous and uniform grossly; 1 cm. high triangular cystic area in center	Bundles of smooth muscle fibers; blood vessels prominent; smaller blood vessels take an active part in tumor
Melnick[1]	48 yr. old woman; duration of tumor 15 yr., with rapid growth last 2 mo.	"Grapefruit size"; 1 cm. from nipple but independent of it	Encapsulated, firm and elastic; replaces entire breast; 2 x 2 x 3 cm. cyst in center	Blood vessel muscular coat blends into tumor; muscle bundles have a perivascular arrangement; thrombosis of blood vessel near cystic area
Lebowitch, R. J., and Lenz, G.: Am. J. Cancer 38 : 73, 1940	58 yr. old woman; duration of tumor 17 yr.; not tender, freely movable	13.8 cm. in diameter; margin 2.5 cm. from nipple	Encapsulated, firm and fibrous; numerous minute cysts on cut surface	Smooth muscle cells with myofibrils and a well vascularized matrix of collagen; few blood vessels in muscle bundles
Stein[5]	54 yr. old woman; duration of tumor 26 yr.; increase of size past 9 yr.; firm, circumscribed, freely movable	Nipple unconnected with tumor mass	Encapsulated; numerous nodules rising above cut surface	Mixed smooth muscle tissue and fibrous tissue; areas of undifferentiated connective tissue
This case	40 yr. old woman; duration of tumor 10 yr.; painful 3 mo.; no rapid change in size, freely movable	10 x 8 x 4 cm. ellipsoid mass; 9 cm. from nipple	Encapsulated, with palpable nodules; numerous nodules rising above cut section	Smooth muscle bundles with fibrous tissue matrix; well vascularized; blood vessels play no direct role in tumor formation

muscular structure of the blood vessels to the tumor proper. In this case the relations of the tumor to its blood vessels or other structures of the breast give no clue to its origin.

SUMMARY

A case of leiomyoma of the breast is described, and the similar cases are gathered from the literature.

7. Strong, L. W.: Am. J. Obst. 68:83, 1913.

PERFORATION OF THE STOMACH OF A NEWBORN INFANT WITH PYLORIC ATRESIA

HAL A. BURNETT, M.D., and BÉLA HALPERT, M.D.

OKLAHOMA CITY

THE PERFORATION of the stomach to be reported occurred in a 2 day old Negro infant in whom atresia of the pyloric portion of the stomach was discovered at necropsy. This is believed to be the seventeenth reported case [1] of perforation of the stomach of the newborn, the third of pyloric atresia and the first of pyloric atresia complicated by perforation of the stomach.

REPORT OF A CASE

A Negro boy was delivered at term by cephalic presentation after episiotomy and use of forceps on March 8, 1947, at 8:09 a. m. Polyhydramnios was present. The child cried spontaneously and appeared well formed. The mother was a 24 year old secundipara, and this had been her fourth pregnancy. The Mazzini test of her blood was negative; the Rh test was positive. At 7 p. m. of the same day the child's abdomen and scrotum became distended; no bowel movements had occurred. Digital examination of the rectum revealed no abnormality. Peristaltic sounds were not heard on auscultation of the abdomen. The child soon became dyspneic. Large amounts of air were discovered in the peritoneal cavity on fluoroscopic examination. At 2 p. m. the next day, exploratory laparotomy disclosed free air and about 60 cc. of turbid liquid in the peritoneal cavity. Near the greater curvature of the stomach there was a necrotic area, 2 cm. in diameter, in which perforation had occurred. The contour of the rest of the stomach was not unusual. The other organs appeared intact. The site of perforation was sutured with interrupted no. 00 chromic catgut, and the abdomen was closed. Supportive treatment, with dextrose and plasma administered intravenously, was of no avail, and the child died the next day, March 10, at 1:45 p. m.

Necropsy (one hour after death).—The body was 50 cm. long and weighed 2,255 Gm. There were no anomalies of the mouth. The neck and the chest were symmetric. The abdomen was slightly distended and somewhat tense. There was a recently sutured incision of the right rectus abdominis muscle, 5 cm. long with a 4 cm. medial transverse extension at the upper end. The scrotum was tense and waterlogged, and 3 cm. in diameter. The testicles were of the usual size.

The peritoneal cavity contained approximately 20 cc. of dark bloody fluid. The anterior surfaces of the liver, the stomach, the spleen, the transverse colon and the omentum were matted together by a fibrinous exudate. There were no anomalous positions of abdominal organs. There was no excess fluid in the pleural or the pericardial cavity. There were no anomalies of the heart and large vessels. Both lungs were air containing throughout.

From the Department of Surgery and the Department of Pathology, University of Oklahoma School of Medicine and University Hospitals.

1. Herbut, P. A.: Arch. Path. **36**:91, 1943.

The stomach measured 6 by 3 cm. In the fundic portion, over the anterior surface, near the greater curvature there was an area of red-brown discoloration, 2 by 1 cm. In the pyloric portion for a length of 1 cm., there was a barely perceptible narrowing to 0.6 cm. in diameter. The mucosal surface was smooth, with the usual gyrations, except over the area of discoloration. In the center of this was the site of perforation, 1 cm. long. In the pyloric portion the lumen was interrupted for a length of 1 cm. The obstruction ended about 1 cm. proximal to the duodenum (figure). The wall of the duodenum was of the usual thickness; its mucosal pattern appeared intact. The loops of small intestine were collapsed and contained green pasty meconium. The colon was contracted and contained a small amount of viscid green liquid. No change was noted on the mucosal surfaces of the small and large intestines and rectum. The left kidney exhibited two complete pelves and ureters with corresponding openings into the urinary bladder. There were no other anomalies.

The segment of the pylorus lacking a lumen. It is about 1 cm. long and ends about 1 cm. proximal to the junction with the duodenum.

Microscopic sections of the stomach from near the site of perforation revealed good preservation of the gastric mucosa. In the tunica propria there were focal extravasations of blood. The submucosa was spread apart and infiltrated by red blood cells. Extensive extravasations of blood were seen in the muscular layers. Externally the area was covered by a hemorrhagic fibrinopurulent exudate. The muscle layers were fairly broad in the uninvolved portion; near the site of perforation they were thin. The appearance was essentially similar in three additional sections from deeper levels of the block.

In a section from the narrow portion of the pylorus, there was no lumen, and the mucosa and the muscularis mucosae were absent. The space was filled by vascular loose connective tissue like that of the submucosa. The appearance was essentially similar in six additional sections from deeper levels. Sections from the duodenum at the level of the papilla of Vater disclosed perfect preservation of the surface epithelium and no changes in the other layers.

COMMENT

Interruption of the continuity of the lumen of the intestine may be due to aplasia, as in Holladay's [2] patient, who lacked a segment 2 cm. long of the pyloric portion of the stomach. It may also be due to the presence of a membranous diaphragm, as in the case reported by Bennett [3] in which a diaphragm obstructed the pyloric portion of the stomach. A third kind of interruption of the lumen may be due to absence of the lumen, mucosa and muscularis mucosae being missing, with the other layers intact. In our case there was true atresia—that is, absence of the lumen of the pyloric portion of the stomach, with the other layers unaffected.

In none of the 16 cases of perforation of the stomach on record was there any distal obstruction.[1] In the 2 recorded instances of obstruction there was no perforation. These circumstances would eliminate mechanical obstruction as the cause of perforation. Congenital focal weakness of the muscular coats of the stomach, observed by Herbut [1] and also in our case, might be the predisposing cause of the perforation.

SUMMARY

Perforation of the stomach of a 2 day old Negro infant with atresia of the pyloric portion of the stomach is reported. The principal factor predisposing to perforation appears to be a congenital defect of the muscular coats of the stomach.

2. Holladay, L. T.: J. Indiana M. A. **39**:350, 1946.
3. Bennett, R. J.: Am. J. Digest. Dis. & Nutrition **4**:44, 1937.

Laboratory Methods and Technical Notes

A FIBRINOGEN-THROMBIN CLOT USEFUL IN TISSUE CULTURE

BENEDICT V. FAVATA, M.D.
ROCHESTER, N. Y.

ONE OF the problems of culturing mammalian cells in vitro is that of insuring uniformity and sterility of the materials used in preparing the tissue and maintaining it in its container. Two substances commonly used are chicken plasma and chick embryo extract. These form the clot used to provide a solid substrate for the growth of the cells.

Chicken plasma is obtained usually by bleeding hens of standard weight and age. Carrel [1] found that the use of plasma from older hens results in poor growth of cultured cells. The plasma must be obtained with great care to prevent clotting. The procedure is tedious and invites many chances of infection. Chick embryo extract is subject to even greater variation and must be used within five to seven days if it is to produce a clot. Earle [2] adds horse serum to the mixture to help prevent liquefaction of the clot, as well as for its growth-promoting properties.

For the past year a mixture of purified fibrinogen [3] and thrombin [3] has been used to form a clot in preparing tissue cultures. The materials are relatively uniform and sterile, and they form a strong clot which will not liquefy for at least six weeks, even when the clot is filled with actively growing fibrocytes.

MATERIALS AND METHOD

The method of producing a fibrinogen-thrombin clot is as follows: The thrombin (labeled: 5,000 units, topical, bovine origin) is used in amounts of 10 mg. in 1 cc. of isotonic solution of sodium chloride as reported by Earle.[2] The purified fibrinogen is used in amount of 50 mg. in 1 cc. of isotonic solution of sodium chloride. The fibrinogen dissolves slowly and stirring should be avoided. The thrombin dissolves easily and quickly. Physiologic balanced solution of sodium chloride may be used instead of isotonic. Porter flasks [4] are used throughout. The body of the flask is 3.5 cm. in diameter and 1.5 cm. thick. Its neck is 16 cm. long and 1.5 cm. in diameter. One part of the thrombin solution added to 4 parts of the fibrinogen solution will form a clot in less than thirty seconds. Place the tissue to be cultured in position, add 4 to 6 drops of the fibrinogen solution and then add 1 or 2 drops of the thrombin solution. Within a few minutes the mixture will have formed a gel, and a supernatant layer of routine nutrient may be added freely. The amounts of the solutions of fibrinogen and

1. Carrel, A.: J. Exper. Med. 18:287, 1913.
2. Earle, W. R., and others: J. Nat. Cancer Inst. 4:165, 1943.
3. This was supplied by Parke, Davis & Company.
4. Porter, K. R.; Claude, A., and Fullam, E. F.: J. Exper. Med. 81:233, 1945.

thrombin may be approximate. Accurate weighing is unnecessary. The thrombin solution remains potent for twenty-four hours. The fibrinogen solution is usually clotted by standing overnight even in the cold. The powdered thrombin and fibrinogen remain effective indefinitely as long as they are kept dry. They can be kept in an incubator at 37 C. to prevent dampness. Care should be taken to prevent the solutions from becoming contaminated with each other, with plasma or with tissue juices, since the thrombin is quickly activated and the fibrinogen clots before it can be used.

The tissues cultured have been chiefly of human origin. They are placed in isotonic solution of sodium chloride and cut into small pieces. Each piece is then transferred to the surface of a single cover slip placed on one side of a Porter flask. A few drops of fibrinogen solution are placed on the piece of tissue, followed by a few drops of the thrombin solution. A few moments later, a few drops of a ' nutrient are placed in the flask, and a mixture of 5 per cent carbon dioxide, 21 per cent oxygen and 74 per cent nitrogen is poured into the flask to replace the air. The nutrient consists of equal parts of Simm's ultra-filtrate of beef serum,[5] citrated human plasma from the hospital blood bank and 9 to 11 day old chick embryo extract in isotonic solution of sodium chloride. The human plasma is allowed to stand in the cold until a cloudy precipitate forms, which is discarded.

The flasks are put in an incubator at 37 C. They are turned once every twenty-four hours. The nutrient is replaced every two to three days.

RESULTS

The clot formed by this method remains clear for at least three weeks. Later fine fibrils may be seen microscopically. The clot does not prevent accurate microscopic observation. The fibrillar network does not hinder the growth of cells. It forms an excellent framework for the preservation of very long processes such as those seen in nerve elements. On the other hand, it can be easily removed so that the cells adherent to the glass are undisturbed and can be preserved and stained.

There is no evidence that the fibrinogen, the thrombin or the ingredients therein are toxic to the cells.[6] Luxuriant growth of glial elements from normal human brain and of cells from normal human testis has been observed within eight hours, and the glass cover slips were covered with cells within forty-eight hours, when the fibrinogen and the thrombin were used in high concentrations. At times the powdered thrombin has been placed directly on the cells without apparent injury, as evidenced by continued proliferation of fibrocytes.

SUMMARY

A method is described for the use of purified fibrinogen and thrombin to form a clot in tissue culture preparations.

5. Simms, H. S., and Sanders, M.: Arch. Path. **33**:619, 1943.

6. Ingraham, F. D., and Bailey, O. T.: J. A. M. A. **126**:680, 1944.

Notes and News

Fourth International Cancer Research Congress.—This congress was held in St. Louis from September 2 to 7, under the joint auspices of the Union Internationale Contre le Cancer and the American Association for Cancer Research. Dr. E. V. Cowdry was president of the congress. Official delegates were present from forty-four countries. Also in attendance were many enthusiastic foreign and domestic guests. Eight general sessions, each addressed by three speakers, and about thirty special sessions, with numerous shorter papers being presented, were held. Sessions were devoted to pathology and diagnosis, chemotherapy, isotopes, radiation biology and therapy, carcinogenic hydrocarbons, cancer genetics, the biology, cytology, chemistry and etiology of cancer, hormones, milk factor, comparative oncology and other subjects. The announcement delivered by telegram to the congress from President Truman that radioactive isotopes had been released for research to qualified foreign research workers was greeted with enthusiasm. Plans were drawn for a permanent world organization to combat cancer. The broad scientific attack on cancer problems which is now under way was apparent, and the announced results were encouraging and stimulating. It is expected that the papers will be published shortly.

Eye Foundation.—At St. Vincent's Hospital, in Los Angeles, Mrs. E. L. Doheny has established the Estelle Doheny Eye Foundation to provide comprehensive ophthalmic laboratory and research facilities. A. Ray Irvine, professor of ophthalmology at the University of Southern California, is chairman of the advisory committee. P. Soudakoff, formerly associate professor of ophthalmology in Peking Union Medical College, is the pathologist on full time.

Cancer Professorship.—Cornell University Medical College has established a professorship of cancer, which has been endowed with $15,000 a year for five years. This represents the first allocation of funds collected in the 1947 campaign of the New York City Committee of the American Cancer Society.

Society News.—The American College of Physicians will conduct its twenty-ninth annual session at San Francisco, April 19 to 23, 1948. General headquarters will be at the Civic Auditorium. Secretaries of medical societies are especially asked to note these dates and, in arranging meeting dates of their societies, to avoid conflicts with the college meeting, for obvious mutual benefits.

The American Association for the Advancement of Science will hold its one hundred and fourteenth meeting, December 26 to 31, in Chicago.

The American Association for the Study of Goiter will meet in the King Edward Hotel, Toronto, Canada, May 6, 7 and 8, 1948.

Research Fellowships.—The American College of Physicians announces that a limited number of fellowships in medicine will be available from July 1, 1948 to June 30, 1949. These fellowships are designed to provide an opportunity for research training either in the basic medical sciences or in the application of these sciences to clinical investigation. They are for the benefit of physicians who are in the early stages of their preparation for a teaching and investigative career in internal medicine. The stipened will be from $2,200 to $3,000. Application forms may be obtained from the American College of Physicians, 4200 Pine St., Philadelphia 4.

Books Received

CHEMICAL CARCINOGENESIS. By Alexander Haddow and others. Price 10 s. London, W.1: Medical Department, The British Council, 1947.

Two numbers (5 and 6 under one cover) of the *British Medical Bulletin* (4:309-444, 1947), a comparatively new publication, are devoted to eighteen reviews under the general title "Chemical Carcinogenesis." Twenty different authors present some of the results of the research supported in part by the British Empire Cancer Campaign over more than twenty years in eleven centers.

Haddow and Kon review the history and the salient features of the chemistry of carcinogenic compounds. Physical methods which have proved highly useful in investigations of carcinogenic hydrocarbons are briefly discussed by Berenblum, Holiday and Jope, who give the limitations of each technic. In a section on the mechanism of carcinogenesis, Haddow critically reviews the modes of action of these chemicals, while Berenblum and Crabtree discuss cocarcinogenesis and anti-carcinogenesis and Dickens delves into the disturbing problem of the influence of the solvent on the carcinogenic response. The metabolism of carcinogenic compounds is reviewed by Boyland and Weigert. In a section on carcinogens of biologic origin, those originating from human tissues are reviewed by Hieger, and the carcinogenic action of heated fats and lipids is critically discussed by Peacock. Under the subtitle of remote carcinogenic action, estrogens are reviewed by Burrows and Horning, stilbene derivatives by Dodds, 2-acetylaminofluorene and related compounds by Bielschowsky, azo compounds by Orr and experimental cancer of the bladder by Bonser. Occupational cancers also receive attention: those of the skin, from Henry; those of the bladder, from Goldblatt, and those due to arsenic, from Currie. In the final section Haddow deals with chemotherapy.

Enumeration of the papers reveals the wide range of subjects covered, but it does not do justice to their high interest or quality. Throughout the papers runs a note of confidence that work with chemical carcinogens is not finished but only beginning and that, however 'productive the past, the future should be brighter. They leave the impression that these men know cancer, understand the enemy and are making their attack wisely.

RECENT ADVANCES IN ENDOCRINOLOGY. By A. T. Cameron, C.M.G., M.A., D.Sc. (Edin.), professor of biochemistry, University of Manitoba Faculty of Medicine; biochemist, Winnipeg General Hospital. Sixth edition. Pp. 443, with 74 figures, including 3 plates. Price $6. Philadelphia and Toronto: The Blakiston Company, 1947.

The previous edition of this compact book was published two years ago. Since that time "no outstanding advance has been made," but there has been steady progress in endocrinology, requiring revisions and additions. The book continues to merit its popularity.

ARCHIVES OF PATHOLOGY

SENSORY NERVES OF THE HUMAN HEART

EDWIN F. HIRSCH, M.D.

AND

JAMES F. ORME, M.D.

CHICAGO

FOR MANY years it has been known that the mammalian heart is supplied with both visceral efferent and visceral afferent nerves. Most of this information has been obtained by studying lower animals. The technical difficulties confronting investigations of the intracardiac distribution of the small nerve trunks and of the individual nerve fibers of the human heart probably account for the fact that there are relatively few studies of the nerves of this organ. The method most widely used to study nerve distribution in lower animals is that of staining with methylene blue as described by Ehrlich, or some modification of it. With this technic the best results are obtained by supravital staining or by staining of tissues immediately after the death of the animal. These conditions are difficult to meet in the postmortem examination of the human body. Various silver impregnation methods have been used, but these are seldom specific enough to differentiate individual nerve fibers from the surrounding connective tissue. With the present popular silver impregnation methods, such as that of Bielschowsky, a nerve fiber can scarcely be identified once it is separated from its trunk, because both nerve and connective tissue fibers are blackened. The silver impregnation method of Cajal as advocated by Nonidez[1] seems to offer some advantages of differentiating the various nerve fibers, particularly the parasympathetic and the sympathetic postganglionic fibers. The former are stained dark brown or black, and the latter yellow or orange, depending on the degree of impregnation. Also stained dark by this method are visceral afferent fibers and preganglionic parasympathetic fibers.

The large nerve trunks of the heart are readily identified and followed in serial sections stained by the usual methods; but these are not adequate for the study of the finer nerve trunks, the individual nerve fibers and the nerve endings. Nettleship[2] stated that Berkley

---

1. Nonidez, J. F.: Am. J. Anat. **65**:361, 1939.

2. Nettleship, W. A.: J. Comp. Neurol. **64**:115, 1936.

in 1894 was the first to suggest that there were sensory nerve endings in the heart. In 1895 Smirnow,[3] using a modification of Ehrlich's methylene blue technic, demonstrated a rich subendocardial plexus of afferent nerves in the auricles of dogs, cats and rabbits. The ventricles contained a similar, though less compact, plexus. A few years later Dogiel [4] demonstrated similar structures in the subepicardial tissues of the hearts of dogs, cats and a child. Woollard,[5] using a modification of Ehrlich's methylene blue stain, studied the efferent and afferent innervation of the heart in snakes, dogs, cats and rabbits. He found all the nonmuscular tissues of the heart to be richly innervated. This included the endocardium, the pericardium, the interstitial connective tissue and the valve structures. Because these tissues have no motor function he concluded that the nerves must be sensory in nature. Myelinated nerve fibers extended for a considerable distance in these structures, and as each fiber approached its termination it gradually lost its myelin sheath. The naked axis-cylinder then extended for a short distance and broke up into a complicated nerve ending. The actual ending was large and showed extensive subdivision, branching and rebranching until huge numbers of filaments were produced. Finally each filament ended in a bulbous expansion. Other observers,[6] using the same method, reported essentially similar afferent nerve endings. Some denied a sensory innervation, and Michailow, quoted by Nettleship,[2] proposed that the cardiac sensory nerve endings have capsules. Most observers have disagreed with this view.

Woollard,[5] Nettleship [2] and others have demonstrated that nerve trunks coming from the subepicardial region follow the course of the coronary arteries through the myocardium to the subendocardial plexus and often form an intricate network around the coronary arteries. Nettleship recognized three nerve plexuses in the heart: (1) those beneath the endocardium, (2) those in the adventitia of the aorta and in that of the pulmonary artery and (3) those in tissues surrounding the coronary arteries. Nettleship [2] investigated the distribution of the various components of the sympathetic and parasympathetic nerves of the heart in cats in which bilateral stellate and middle cervical sympathetic ganglionectomy, bilateral vagotomy, vagotomy proximal to the nodose ganglion or bilateral removal of the first five thoracic dorsal root ganglions had been performed. Following bilateral stellate and middle cervical ganglionectomy, the ventricular trunks and the epicardial network of the ventricles degenerated whereas similar structures of the auricles escaped. In addition, that portion of the endocardial

3. Smirnow, A.: Anat. Anz. **10**:737, 1895.
4. Dogiel, A. S.: Arch. f. mikr. Anat. **52**:44, 1898.
5. Woollard, H. H.: J. Anat. **160**:345, 1926; Heart **13**:319, 1926.
6. Nettleship.[2] Smirnow.[3] Dogiel.[4]

plexus near the apex of the ventricles, simple nerve endings in the fat and from one half to three fourths of the coronary artery plexus degenerated. The animals whose thoracic dorsal root ganglions had been removed had changes similar to those observed in animals which had undergone bilateral stellate and middle cervical sympathetic ganglionectomy except that the degeneration of the nerve trunks of the heart was much less. Some of the larger fibers of the coronary artery plexus and the portion of the endocardial plexus near the apex degenerated. With total vagotomy the greater part of the nerve fibers in the trunks of the epicardial plexus of the ventricles was spared. Atrial trunks had fragmentation and degeneration of the largest myelinated fibers. The endocardial, aortic and pulmonary artery plexuses similarly degenerated. With section of the vagus nerve proximal to the nodose ganglions, the myelinated fibers of the auricles were spared, but the fibers terminating about the epicardial ganglions degenerated.

Accordingly, in lower animals the stellate and cervical sympathetic ganglions supply the major portions of the efferent fibers to the epicardial network of the ventricles and to the portion of the endocardial plexus near the apex of the ventricles. The thoracic dorsal root ganglions supply afferent fibers to the ventricles and some of the myelinated fibers to the coronary arteries. The nodose ganglions of the vagus nerves supply afferent fibers to all of the endocardial plexus except the apical portion and to the aortic and pulmonary artery plexuses. The motor nucleus of the vagus nerve supplies the preganglionic fibers which are distributed to the epicardial ganglions.

MATERIAL AND METHODS

Blocks of tissue from the heart of 1 infant and 3 adults were studied. The infant tissue included portions of the left auricle and the left ventricle. All of the adult tissues were from the left ventricle along the course of the anterior descending branch of the left coronary artery. None of these blocks included tissue closer than 2 cm. from the origin of the artery mentioned. From one adult heart five serial blocks were taken, beginning at a level 2.5 cm. from the origin of the anterior descending branch of the left coronary artery. The first block was 1 cm. long as measured parallel to the coronary artery and about 1.5 by 1 cm. in the other dimensions. This block was fixed in formaldehyde solution, and sections cut from it by the freezing method were stained for myelin by the Spielmeyer method.[7] The second block was about 8 mm. long and was fixed in Bouin's fluid for seventy-two hours, after which it was embedded in celloidin (a concentrated preparation of pyroxylin) and paraffin in the manner suggested by Bensley and Bensley.[8] Serial sections, 8 microns thick, were cut

7. Mallory, F. B.: Pathological Technique, Philadelphia, W. B. Saunders Company, 1938.

8. Bensley, R. R., and Bensley, S. H.: Handbook of Histological and Cytological Technique Chicago, University of Chicago Press, 1938.

from this block, and every third slide was stained with the modification of the Masson-Goldner trichrome stain recommended by Foot.[9] When indicated, every slide was stained by this method or with the silver impregnation method of Biel-schowsky.[7] The third block was cut about 3 mm. thick, was fixed in alcoholic chloral hydrate solution and was impregnated with silver according to the method described by Nonidez.[1] The fourth and fifth blocks corresponded in size and preparation to blocks 2 and 1, respectively. One block of tissue from an adult heart was fixed in Zenker's solution and embedded in paraffin, and serial sections, 8 microns thick, were stained with the trichrome method mentioned. Three blocks of tissue, about 2 mm. thick, from an adult heart were stained by a modification of Ehrlich's methylene blue method for nerve fibers.[7] These blocks were embedded
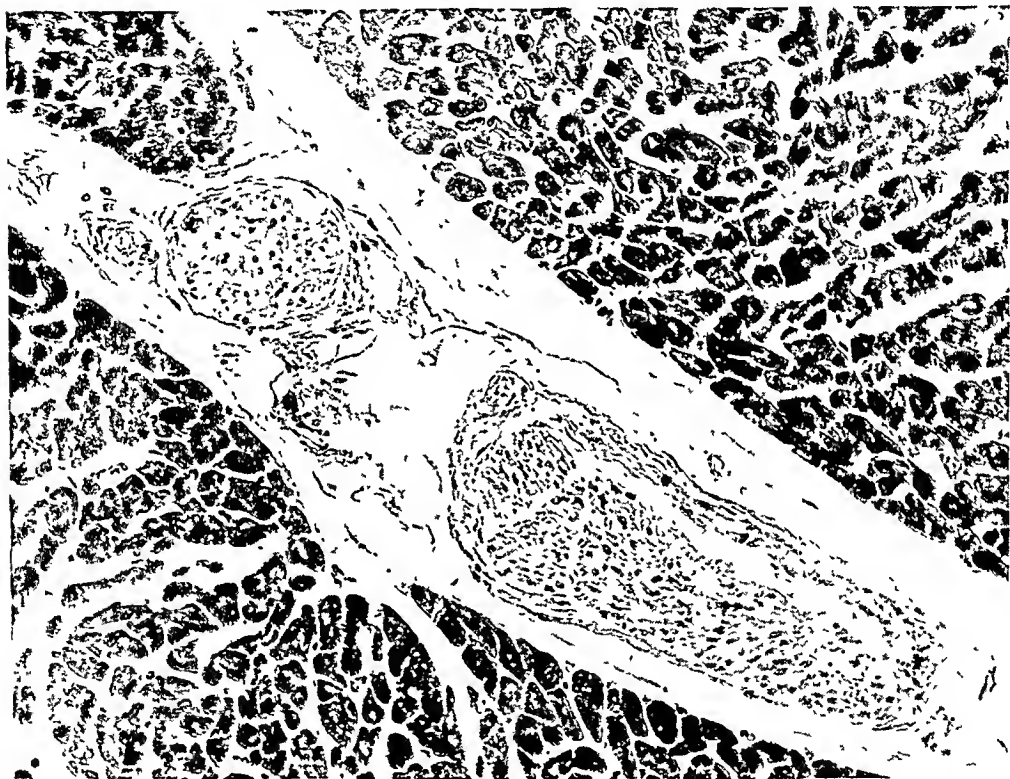
Fig. 1.—Photomicrograph of nerve trunks in the periarterial connective tissues between bundles of cardiac muscle. × 198.

in paraffin and sectioned. All sections were cut at right angles to the course of the coronary artery.

OBSERVATIONS

In the histologic sections prepared by each method, nerve trunks of varying sizes were readily identified and could be traced in serial sections through the block of tissue. The subepicardial regions had a large number of nerve trunks, many just beneath the serosal surface in the fibrous and fat tissues. The branches of the coronary artery were

9. Foot, N. C.: Pathology in Surgery, Philadelphia, J. B. Lippincott Company, 1945.

accompanied by nerve trunks of varying sizes, and these trunks accompanied the arteries through the myocardium to the subendocardial regions (fig. 1). Often more than one nerve trunk accompanied an artery, and the position of the nerve in relation to the artery varied. At times it was considerably removed and was in the loose areolar tissue, but at other levels it was in the compact fibrous adventitia of the artery. When in the latter position, the nerve trunk was usually small and seemed to end eventually in the adventitia of the artery. The nerve trunks often branched to follow the divisions of the artery, and sometimes a nerve trunk passed from an artery at almost a right angle,
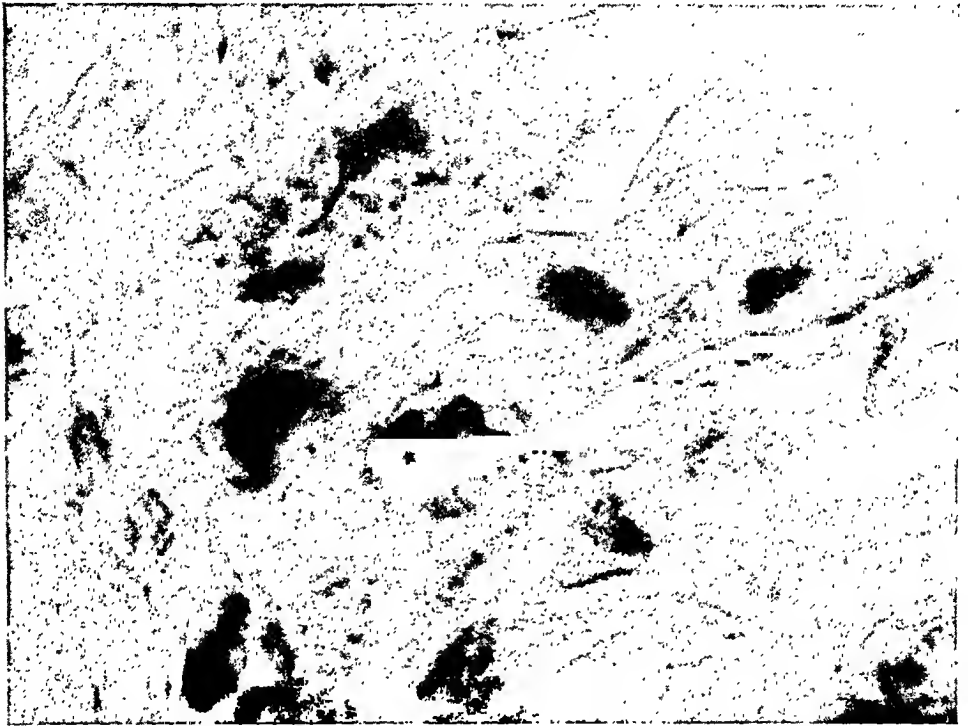
Fig. 2.—Photomicrograph of the terminal portion of a myelinated fiber in the adventitia of an artery. × 1360.

extended in loose areolar tissue between bundles of cardiac muscle and became associated with another blood vessel. The size of the nerve trunks gradually decreased toward the endocardium.

With sections prepared by freezing formaldehyde-fixed tissue and staining for myelin, nerve fibers with myelin sheaths were readily demonstrated in all blocks of tissue so prepared. The fibers varied in diameter, but most of them would be considered thick. Many had small varicose swellings along their course, and nodes of Ranvier were observed. Myelinated fibers were readily distinguished in nerve trunks and extended from the trunks into the surrounding stromal tissues or to

the adventitia of an artery, where they seemed to end. Myelin sheaths disappeared before the nerve terminated, but often a sheath could be traced into the adventitia of a blood vessel (fig. 2).

The silver impregnation method advocated by Nonidez[1] clearly demonstrated the nerve trunks. Most of the fibers were stained yellow, as were the surrounding fibrous and muscle tissues. A few large fibers were stained dark brown or black in the manner Nonidez ascribed to postganglionic parasympathetic or afferent nerve fibers (fig. 3). The heavily stained fibers extended from the nerve trunks into the surrounding connective tissue, but structures thought to be nerve endings as

Fig. 3.—A nerve trunk with many myelinated fibers in perivascular connective tissues deep in the myocardium. × 346.

described by Nonidez[10] were not observed. A few sections of the tissues fixed in Bouin's solution were stained according to the Bielschowsky method, but this was abandoned because individual nerve fibers could not be distinguished from the impregnated reticulum.

Nerve ganglion cells were observed in the epicardial tissues of the auricle of the infant's heart, but none was observed in the tissues of the ventricle. This is in agreement with the studies made by Woollard[5] and Nettleship[2] in lower animals but is in contrast to the studies of

---

10. Nonidez, J. F.: Am. Heart J. **26**:577, 1943.

animal tissues made by Dogiel and Smirnow. In the limited investigation with the methylene blue technic, the tissues stained irregularly, and while nerve trunks and nerve fibers could be identified, nerve endings of the type described were not observed. The same is true of the silver impregnation method of Nonidez.[1] Small clusters of black silver compounds were distributed in the tissues at some levels, but these were not definite enough to be considered sensory nerve endings. Encapsulated nerve endings were not observed in any of the tissues. This is in agreement with the studies made in lower animals by Nettleship.[2]

The presence of myelinated nerve fibers in the heart in regions not requiring motor function, as well as their structure, indicates that they are afferent. Woollard[5] has described myelinated postganglionic parasympathetic fibers arising from the intracardiac ganglions. However, most of these fibers lose their myelin sheaths after a short course and are distributed to the musculature and the conduction system of the auricles. Kuntz[11] stated that some gray rami communicantes of the sympathetic system contain myelinated fibers. However, given a myelinated nerve fiber of the ventricle of the heart which extends into and seems to end in fat or fibrous connective tissue, the assumption is reasonable that such a fiber is afferent in function. Such fibers are widely distributed in the subepicardial and subendocardial tissues and in the perivascular connective tissues of the myocardium.

COMMENT

Many studies of the distribution of the nerves of the hearts of lower animals have been made. In some, portions of the nerve supply have been interrupted, and the subsequent areas of degeneration indicated specific nerve supplies of various regions of the heart. Such studies of the nerve supply of the human heart are not feasible. The anatomic relations of the sensory nerves of the human heart have great interest, because these fibers are the medium for transmission of the pain sensations that occur with various forms of heart disease, notably angina pectoris, coronary occlusion and acute myocardial infarction. However, only a few reports of such studies of the human heart have been published. According to Gorham,[12] the myocardium itself is insensitive, but the heart as a structure has numerous afferent (sensory) fibers that respond to painful stimuli. These fibers are in the adventitial layer of the coronary arteries and send terminal branches at intervals to the smooth muscle of the media. This sensory nerve system, Gorham stated,

11. Kuntz, A.: The Autonomic Nervous System, Philadelphia, Lea & Febiger, 1945, pp. 48-49.

12. Gorham, L. W.: A. Research Nerv. & Ment. Dis., Proc. (1942) **23**:337, 1943.

is basic in any discussion of the mechanism of cardiac pain. The pain-receptive structures in other parts of the body (cornea, Tower[13]; skin, Gasser[14]) are nerve fibers, a large portion myelinated but losing their sheaths in the terminal branches. Gasser observed both myelinated and nonmyelinated fibers in sensory nerves, the proportion of each ranging widely.

The myelinated fibers observed in nerves distributed along the coronary arteries of the heart are considered to be afferent and hence sensory in function. No conclusions are possible as to whether these afferent nerve fibers are part of the vagus nerve, with their cell bodies in the nodose ganglions, or whether they accompany the sympathetic nerves and have their cell bodies in the dorsal root ganglions of the spinal cord. These visceral afferent (sensory) fibers in the nerves of the human heart are the anatomic structures by which sensory impulses are transmitted to the central nervous system, and, as Gorham[12] has stated, they are basic in any discussion of the mechanism of cardiac pain. If a theoretic nonmedullated sensory fiber component, not determined by these anatomic studies, is present, the arterial and periarterial tissues could have a much larger sensory supply than the number of myelinated fibers would indicate. Our study does not determine which tissues of the ventricle of the human heart have the largest supply of afferent nerves, but other reports state that the base of the heart has the richest supply of nerves.

The changes of atherosclerosis are usually in the first portion of the coronary arteries, that is, at the base of the heart, where the nerve supply is greatest. The arteriosclerotic changes occurring in the wall of the artery seem not to initiate the painful sensations associated with coronary disease, at least not the severe form. Although the exact nature of the stimulus producing pain in these sensory nerves of the heart is not fully understood, by general agreement this stimulus seems to be associated in some way with impedance or suppression of the coronary blood flow. A popular view holds that the pain is caused by relative ischemia or anoxemia of the cardiac muscle. If the studies which indicate that the myocardium itself is insensible to painful stimuli are valid, this explanation of the origin of the painful sensation has little to support it anatomically except in an indirect way. Lewis[15] has proposed that the ischemic muscle liberates a factor, "P," which diffuses into the tissues and there stimulates the nerve endings. Others have proposed that acid metabolites, such as lactic

13. Tower, S. S.: A. Research Nerv. & Ment. Dis., Proc. (1942) **23**:16, 1943.

14. Gasser, H. S.: A. Research Nerv. & Ment. Dis., Proc. (1942) **23**:44, 1943.

15. Lewis, T.: Arch. Int. Med. **49**:713, 1932.

acid, which accumulate during ischemia provide the stimulus for the sensation of pain. Moore and Singleton [16] observed a pain response in dogs when lactic acid was injected into the coronary circulation, and have formed a theory concerning cardiac pain because a concentration of lactic acid approaching a pain-producing threshold could be demonstrated in blood flowing from the coronary sinus with fatigue of the heart muscle. Moore and Greenberg [17] emphasized lactic acid as the tissue factor stimulating painful sensations in circulatory disturbances of the heart. Doubtless small concentrations of many other definite chemical substances injected into the coronary circulation would cause similar painful sensations.

The myelinated nerves distributed in the fibrous tissues along the coronary arteries and the terminal branches extending into the walls of these vessels suggest, from anatomic considerations, that stimuli for pain develop in the tissues in and about these arteries. Katz, Mayne and Weinstein [18] studied ischemia as a cause of pain experimentally in dogs and concluded that the sensory response is due not to occlusion of the coronary artery and interference with the intra-arterial flow of blood but to direct stimulation of afferent fibers in the nerve plexus surrounding the coronary vessels. Gorham [12] produced cardiac pain in dogs by making tension on the coronary arteries without interfering with the circulating blood, that is, ischemia. These results led him to suggest that tension of the tissues in and about the coronary arteries is the stimulus for cardiac pain. Supporting evidence is derived from the observation that occlusion of the lumen of a normal peripheral artery, such as a femoral artery, is associated with pain and that when the obstruction is sudden the pain is severe and at the site of obstruction. The excruciating pain associated with the tearing of the tissues by a dissecting aneurysm of .the aorta is well known and is a diagnostic clinical symptom. Here the trauma done to tissues rather than anoxemia is regarded as the stimulus of the pain, and more clearly we realize that injuries of the arterial tissues cause pain and that, as in other parts of the arterial system, the aorta and the arteries (or veins) have nerves with sensory fibers. Woollard's studies of the innervation of peripheral blood vessels led him to comment that the endings of myelinated nerves (sensory) have been observed in the walls of blood vessels by many authors but that these observations seem to have gained little credence, being either ignored in the interpretation of vascular disturbances or, if admitted, regarded as subserving only a

16. Moore, R. M., and Singleton, A. O.: Proc. Soc. Exper. Biol. & Med. **32**:1492, 1935.

17. Moore, R. M., and Greenberg, M. M.: Am. J. Physiol. **118**:217, 1937.

18. Katz, L. N.; Mayne, W., and Weinstein, W.: Arch. Int. Med. **55**:760, 1935.

minor sensory function. As early as 1905, according to Woollard,[5] Lapinsky described five types of nerve endings of medullated nerves in blood vessels: (1) those of myelinated fibers which lose their sheaths at some distance from their termination, to end finally in freely branched twigs; (2) branched endings forming a terminal brush of considerable length; (3) bulbous thickenings of the ends of small twigs given off at right angles from medullated nerves; (4) small fibers ending abruptly in a granule; (5) undetermined forms, in which branches of medullated nerves appear to end in large oval swellings. Woollard, from his own observations on the endings of medullated (sensory) nerves in and about blood vessels, stated that some are pacinian corpuscles, while others end freely in adjacent fat and connective tissues and in long, extended twigs in the adventitia of the vessels. Woollard observed that medullated fibers were most abundant on the peripheral vessels and on this basis concluded that puncture or ligation of these was more likely to be painful than that of large vessels. Gammon and Bronk[19] observed the initiation of afferent impulses in sensory nerves of the mesentery, which increased in number and frequency with distention of the mesenteric blood vessels at pressures above a critical level. A decrease of the caliber of the vessels caused a decrease of the afferent impulses.

These references to the sensory innervation of systemic arteries do not include all pertinent reports. Studies of the distribution of the sensory nerves of the heart demonstrate that here, also, the fibers are arranged in close association with the coronary arteries. This pattern of sensory nerve supply common to the peripheral and the coronary arteries, with perhaps some specific adaptations in the heart, provides an anatomic structure in which comparable principles may be noted. There is no dispute concerning the fact that various physical or chemical stimuli can arouse painful sensations in tissues; the recognition of the specific component with each stimulus depends on how minute the analysis can be. Sudden occlusions of peripheral arteries are painful by common clinical observation, whether caused by tension or actual injury of the tissues. No one thinks of this immediately painful experience in the general terms of anoxemia. Coronary arteries, as disclosed by anatomic studies, have a sensory nerve supply similar to that of the peripheral arteries. Accordingly, in each category painful sensations can occur with comparable stimuli. Since in the heart the sensory nerves are distributed mainly along the coronary arteries, and thrombosis usually occurs in the proximal segments of these arteries, the stimuli producing cardiac pain in vascular accidents of the heart would seem to arise, at least from anatomic considerations, not in the

19. Gammon, G. O., and Bronk, D. W.: Am. J. Physiol. **114**:77, 1935.

myocardium but in the arterial and periarterial tissues and in a manner entirely like that noted with sudden thrombosis of a peripheral artery. This view of the site of origin of the pain attending cardiac disease may seem novel, but actually it brings present concepts of the origin of the painful cardiac sensations of coronary disease and other cardiac disorders into a common pattern, applicable with comparable injuries or stress to both the coronary and the peripheral arteries. This concept also focuses attention not on the heart as a unit structure but on its sensory components and their significant relation to the coronary arteries.

SUMMARY

Myelinated (sensory) nerve fibers are distributed along the coronary arteries and their branches in the myocardium. Their terminal divisions are filaments without myelin sheaths, some of which extend directly into the walls of the arteries.

These sensory fibers are the anatomic pathway by which painful sensations arising in the heart are transmitted to the central nervous system.

The observation that these fibers are distributed along and in the walls of the coronary arteries suggests that in the heart the painful stimuli arise in and about the coronary arteries rather than in the muscle tissues.

Sensory fibers are distributed to the walls of peripheral arteries in a pattern quite similar to that observed with the coronary arteries of the heart. Accordingly, this common pattern of distribution of the sensory nerves of both the coronary and the peripheral arteries provides a common anatomic basis for the reception of pain sensations aroused by comparable conditions in all arteries of the body.

The tissue factors arousing the pain sensation associated with an injury or with thrombosis are similar for all arteries, the response depending on the sensory nerve supply.

FOCAL ANEMIA, LEUKOCYTOSIS AND FATTY INFILTRATION OF THE LIVER (SO-CALLED SEPTIC LIVER SPOTS)

FRANZ WENGER, M.D.

MARACAIBO, VENEZUELA

E VERY pathologist is familiar with certain yellowish spots frequently seen on the surface of the liver, which stand out clearly against the surrounding tissue. For the first time, this lesion was mentioned briefly in 1904, by Beneke,[1] who stated that in cases of acute lesions of the brain, acute peritonitis and other diseases accompanied by shock there occur in the liver characteristic wedge-shaped anemic foci; he explained them as anemia due to vasomotor spasm occurring during agony. Five years later Helly[2] gave a thorough macroscopic description. He distinguished two kinds of liver spots: The first are somewhat diffuse and always superficial, and their shape and localization indicate their origin—a postmortem change due to pressure of the ribs or of the abdominal viscera. The second have sharp, although irregular, outlines and are grouped around the branches of the portal vein. By microscopic observation of this second group of liver spots he found basophilia of the hepatic cells (in sections stained with methylene blue and eosin), focal edema and anemia, and he explained the striking yellowish color of the lesions as being due to diffuse fatty degeneration of the whole organ, which inside the spot might be more visible because of the anemia. Helly expressed the belief that the focal edema was prior to the focal anemia, which in his opinion is produced by the compression of the sinusoids, perhaps through cloudy swelling of the liver cells. At any rate, he said, the cause of the lesion must be looked for in toxic matters, circulating in the blood during acute sepsis; for that reason, and because he had found the spots only in cases of sepsis, he called them "septic spots."

More recently Roessle,[3] speaking of hepatitis in general, considered these spots as localized serous hepatitis and stated that no sharp limit can be drawn between the two forms of serous hepatitis, the localized

---

From the Department of Pathology, Tumor Clinic.

1. Beneke, R.: Deutsche med. Wchnschr. 30:1489, 1904.

2. Helly, K.: Verhandl. d. deutsch. path. Gesellsch. 13:312, 1909.

3. Roessle, R.: Schweiz. med. Wchnschr. 10:4, 1929.

and the diffuse.   On the other hand, Gerlach [4] found the "spots" on the surface and deep in the parenchyma, and they were well pronounced in cases of sepsis, especially after abortion; but he saw them also in nonseptic conditions, sometimes associated with cerebral lesions.

The references cited are, as far as I can see, all that are to be found in the literature on this subject—which seems to have been badly neglected.   This neglect is the more astonishing even in view of the fact that the descriptions given in the foregoing paragraph are quite inadequate in the light of my experience, especially in regard to the microscopic structure; only to Helly's segregation of spots due to post-mortem compression should everybody fully agree.

From my material of 32 cases, used for the following report, such postmortem changes were carefully excluded.   Among the causes of death in these cases there was a slight prevalence of acute peritonitis, sepsis and cerebral lesions as compared with the frequency of the diseases in this autopsy population.   During this investigation two types of lesions, quite different, especially in their microscopic appearance, were found to occur, which formerly certainly have been listed together as "septic liver spots."   They will be described separately as types A and B.

GROSS APPEARANCE

Type A (26 cases).—The lesions of type A were found nearly always on the anterior surface of the liver, more frequently on that of the right lobe than on that of the left, and generally near to the insertion of the ligamentum falciforme. The size of these spots varied greatly, from 5 mm. to 7 cm. in diameter.   Their shape usually appeared to be irregularly polygonal.   The color was yellowish and always clearer than the rest of the liver surface.   The limits were sharp all around, or were sharp on one side and shaded away into the rest of the surface on the other.   Frequently the sharp limit was produced by a dark zone of hyperemia around the spot.   On the cut surface the parenchyma beneath the spot was also yellowish; the depth of this clear zone rarely exceeded 1 cm.; only exceptionally it was wedge shaped, its form being generally quite irregularly flat. Some livers showed several spots of the type here described.

Type B (6 cases).—In 4 cases the spots were exactly on the inferior border of the liver, nearly in the notch where the round ligament inserts, whereas in a fifth there was a spot on the under surface.   They were regularly round and 1 cm. in diameter.   There was no hyperemia of the surrounding tissue.   On the cut surface they were wedge shaped, and a big portal space could be distinguished on the point of the wedge.   One lesion of this type was associated with another round spot on the under surface of the liver, where a fibrous adhesion inserted, coming from the gallbladder.   On the cut surface the part of the parenchyma belonging to this second spot was not wedge shaped but merely flat.   In the last

---

4. Gerlach, W., in Henke, F., and Lubarsch, O.:   Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1930, vol. 5, pt. 1, p. 103.

case of this group the under surface of the liver showed several clear spots, very irregular, measuring up to 5 mm. in diameter, like splashes.

MICROSCOPIC APPEARANCE

Type A.—The sharp limits of the clear areas which were so conspicuous to the naked eye frequently disappeared after the staining was done, and I used to confront the stained slides with the paraffin blocks to get oriented quickly. Orientation was easier when the hyperemia of the margins was marked. The common feature of all the clear areas of hepatic parenchyma which belonged to this group was the anemia, with the sinusoids practically devoid of red cells. In the cases of anemia alone the sinusoids were empty or contained some slightly eosinophilic amorphous matter, apparently coagulated albuminous fluid. In some cases this eosinophilic matter was found also outside the sinusoids, in the spaces of Dissé. One case showed the sinusoids under the liver spot filled by slightly eosinophilic round corpuscles, which contrasted strongly with the bright red color of normal red blood cells; they were probably red cells from which the hemoglobin had been extracted (by stasis?).

In only 3 cases, in which the liver in general was more or less normal, were all the signs of Roessle's serous hepatitis visible beneath the clear spot, i. e., edema fluid in the spaces of Dissé, dissociation of hepatic cell cords, necrosis of hepatic cells, especially around the central veins and others. Usually, when serous hepatitis was found, none of the signs of injury was more pronounced inside the clear area than in the rest of the parenchyma. Yet the local anemia seemed to make more visible such damage of tissue, so that it stood out more clearly in the slide, and at a rapid examination the anemic area feigned a more severe injury when, as a matter of fact, the same degree of injury was present .in the whole organ. Fatty infiltration was never so prominent as to be responsible for the clear color of the area, but in many cases it was heavier under the clear spot than elsewhere. I could never find Helly's basophilia of the protoplasm of the hepatic cells, even when employing Mallory's eosin and methylene blue stain.

In 11 cases the leukocytosis of the area was striking, the area contrasting in this respect with the rest of the parenchyma, where leukocytes were found with no more frequency than usual. In these cases the sinusoids were crammed with polymorphonuclear leukocytes, lymphocytes and large mononuclear cells, and such cells were found also outside in the pericapillary spaces. This dense leukocytosis was sometimes so well pronounced that practically all the sinusoids of the clear area were filled with white blood cells, just as they are being filled with red cells (fig. 1). In other cases the leukocytosis was more patchy in distribution, being localized especially under Glisson's capsule and in the peripheral zone of the clear areas, and here the leukocytic sinusoids were sometimes the immediate continuation of the sinusoids of the hyperemic margin, which were crowded with erythrocytes (fig. 2). Sometimes a case was classified at first as one simply of anemic or of "serous" hepatitis, and only after cutting several blocks of tissue a small leukocytic area could be found. It is noteworthy that this leukocytosis of the sinusoids was not at all related to injury of liver cells (fig. 3) or other signs of hepatitis, being seen in livers otherwise practically normal, as well as in livers showing severe damage. Outside the clear area I could never find sinusoids crowded to such a degree by leukocytes.

Type B.—Just as in their gross appearance, the lesions which belonged to this group were quite uniform in their histologic structure. Their limits were perfectly

sharp and ran more or less along the portal spaces. The liver cells were infiltrated by big droplets of fat, which filled the whole body of the cell; only around the portal spaces some few hepatic cells without fat could be found (figs. 4 and 5). The whole area was rather anemic, but there was no marked leukocytosis. In 1 case a few minute foci of necrosis were seen, with secondary leukocytic infiltration. In another case the fatty infiltration was produced by tiny droplets, and not by big ones. The branches of the portal vein and of the hepatic artery which ran toward these areas of fatty infiltration appeared to be normal.
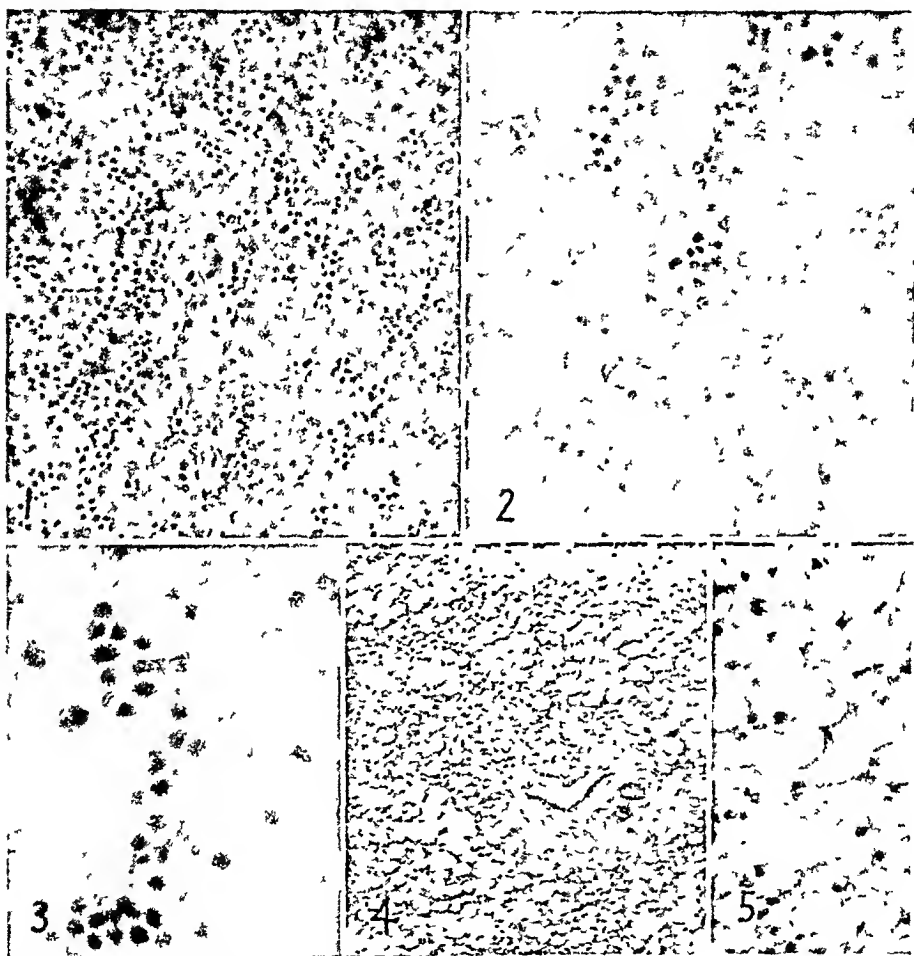
Fig. 1.—Focal leukocytosis of the liver. × 180.

Fig. 2.—Border of leukocytic focus; sinusoids are shown, which are filled on one side (top) by leukocytes and on the other side (bottom) by red blood cells (belonging to the hyperemic margin). × 365.

Fig. 3.—White blood cells filling the sinusoids, the lining hepatic cells are normal. × 690.

Fig. 4.—Focus of fatty infiltration, sharply limited against the adjoining normal liver tissue. × 24.

Fig. 5.—Liver cells heavily infiltrated by big fat droplets inside the focus. × 330.

COMMENT

The findings in the lesions which generally are called "septic spots" are both surprising and difficult to explain. In the cases of type A the most prominent and only common feature was the severe local anemia, which certainly determined by itself the clear color of the area. As pointed out already by Beneke,[1] such severe anemia is incompatible with life, and as there is no necrosis, the anemia cannot have started before agony. As no obstruction ever was found in the pertinent blood vessels, a spastic contraction of these vessels is supposed to exist, although it should be remembered that two different sets of vessels, the branches of the hepatic artery and those of the portal vein, have to close their blood supply simultaneously. Still little is known about the finer structure and function of the circulatory system of the liver; only recently Freerksen[5] and especially Coronini[6] complemented Pfuhl's[7] earlier report of particular musculoelastic structures in the liver arterioles which certainly regulate the blood pressure in the capillaries for proper mixture of arterial and venous blood. Transitory dysfunction of such a mechanism might result in the local anemia here described.

Roessle's[3] view that the "septic spots" represent just local serous hepatitis is based principally on the findings of edema fluid in Dissé's spaces; this, as well as the other signs of serous hepatitis, was only rarely seen in any significant degree in the foci here described, so as to contrast with the remainder of the parenchyma. And where such a difference is seen between the liver structure inside and that outside the clear area, it is explained more easily as an injury from prolonged anemia, occurring perhaps when agony lasted sufficiently long to permit the development of such changes.

To find dense accumulations of leukocytes in these areas was most surprising. Of course, the suggestion that this must be a severe inflammation was near at hand, but had to be rejected. In diffuse hepatitis, such as that in catarrhal jaundice (infectious jaundice), leukocytic infiltration is not conspicuous,[8] although some authors[9] have mentioned it.

5. Freerksen, E.: Klin. Wchnschr. **22**:733, 1943.

6. Coronini, C.: Zentralbl. f. allg. Path. u. path. Anat. **82**:241, 1944.

7. Pfuhl, W., in Möllendorf, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1932, vol. 5, pt. 2, p. 235; cited by Coronini.[6]

8. (a) Gaskell, J. F.: J. Path. & Bact. **36**:257, 1933. (b) Barber, H., and Osborn, G. R.: ibid. **49**:581, 1939. (c) Siegmund, H.: München. med. Wchnschr. **89**:463, 1942.

9. (a) Klemperer, P.; Killian, J. A., and Heyd, C. G.: Arch. Path. **2**:631, 1926. (b) Schrumpf, A.: Ann. d'anat. path. **9**: 17, 1932. (c) Roholm, K., and Iversen, P.: Acta path. et microbiol. Scandinav. **16**:427, 1939. (d) Roulet, F.: Virchows Arch. f. path. Anat. **310**:436, 1943. (e) Lucké, B.: Am. J. Path. **20**: 471, 1944. (f) Roessle.[3]

Wohlwill [10] described a form of diffuse hepatitis which was accompanied from the beginning by leukocytic infiltration just as any other inflammation elsewhere in the body. The closest approach to my findings was that presented by Dible, McMichael and Sherlock,[11] who in cases of hepatitis of different causes saw in the sinusoids a general enlargement and hyperplasia of "endothelial" cells, giving the hepatic lobule an abnormally cellular appearance; they even described small focal accumulations of cells, including leukocytes; but this as well as all the other reports relate the cell accumulations to degeneration or necrosis of liver cells, whereas in several of my cases the liver cells seemed to be perfectly normal, or cell degeneration was in no relation in degree or localization to leukocytosis. Bacterial stains were always negative. Moreover, the number of cells inside and outside the sinusoids exceeded very much that of all the reports cited in the foregoing pages.

It is for the moment impossible to give a satisfactory explanation for this curious local leukocytosis. But I wish to point out that the leukocyte content of the liver, as that of some other organs, is known to vary greatly and to be quite independent of inflammation and of the leukocyte content of the peripheral blood. Gräff [12] described capillaries which were completely filled with leukocytes, with only occasional red blood cells between them, and he supposed that such accumulations of leukocytes in the inner organs may even influence inversely the leukocyte content of the peripheral blood. Hino,[13] after extensive anatomic studies, concluded that the only possible cause for this accumulation of leukocytes is the number and the length of the capillaries of some inner organs, especially of the liver, the kidney and the spleen, and that the leukocytes adhere to the vessel walls because of the slowing down of the blood stream. In my cases of severe local anemia such slowing down must have been marked, and if Hino's interpretation of the general leukocytosis of inner organs is correct, the local accumulation of white cells in anemic areas should be simply mechanical, due to the stasis, and without any relation to chemotaxis or similar phenomena.

While the simply anemic and the leukocytic foci of type A seemed not to be fundamentally different lesions, as there were borderline conditions of only limited leukocytic infiltration, the lesions of type B, the fatty infiltrations, constituted a second group, totally different from the first, and there was no bridge between them. These fatty foci have certainly a different causation and pathogenesis, and their shape and the close relation (macroscopic and microscopic) to the bigger branches of blood vessels point clearly to an embolic origin, although this investiga-

10. Wohlwill, F.: Schweiz. Ztschr. f. allg. Path. u. Bakt. **2**:240, 1939.
11. Dible, J. H.; McMichael, J., and Sherlock, S. P. V.: Lancet **2**:402, 1943.
12. Gräff, S.: Berl. klin. Wchnschr. **58**:84, 1921.
13. Hino, J.. Virchows Arch. f. path. Anat. **256**:30, 1925.

tion could not disclose the nature of the emboli. Very similar foci of severe local fatty infiltration were observed by Menkin [14] in dogs after intravenous injection of necrosin, a substance contained in the euglobulin fraction of purulent exudate, which seems to be responsible for the tissue damage in typical acute inflammations. I could not find any other reference to focal fatty infiltration of the type here described.

The name given to these clear areas by Helly,[2] which still is widely in use, may safely be abandoned. First, these lesions are not spots; they are tridimensional. Second, there exists no relation to septic diseases, as they occur in many nonseptic diseases. Further studies are necessary to cast light on their causation and pathogenesis. In the meantime, purely descriptive names should be used, which take into account the histologic aspects of the lesions, such as "focus of anemia" and "focus of fatty infiltration," respectively.

SUMMARY

In a series of cases of so-called septic spots of the liver, two quite different types of lesions were observed. In the first the parenchyma under the spot was severely anemic, this lack of red blood cells being responsible for the clear color of the whole area. In many cases there was in addition to the anemia a dense accumulation of polymorphonuclear leukocytes, lymphocytes and large mononuclear cells inside and outside the sinusoids.

The second type, in most instances, was wedge shaped on the cut surface and sharply limited; it showed severe fatty infiltration of the liver cells.

No satisfactory explanation can be given as to the causation and genesis; anyhow, an inflammatory origin can be ruled out probably for both groups of lesions; in regard to the first, all the findings, including the local leukocytosis, might be explained by vascular spasm and consecutive slowing down of the blood stream during agony. The second group seems to have an embolic origin. As both types of lesions are associated with a great variety of diseases, the name "septic spots" should be substituted by "foci of anemia" or "foci of fatty infiltration," respectively.

---

14. Menkin, V.: Arch. Path. **36:**269, 1943.

PATHOGENICITY OF BAGASSE

B. GERSTL, M.D.
HARTFORD, CONN.

M. TAGER, M.D.
NEW HAVEN, CONN.

AND

N. A. MARINARO, M.D.
HARTFORD, CONN.

SUGAR CANE from which the sugar content has been extracted is called bagasse. This material is stored in the open for months and even years; then it is broken, processed and finally pressed into various shapes yielding building and insulating boards, such as "celotex." Workers breaking bagasse sometimes acquire a respiratory disease characterized clinically by cough, dyspnea, occasional hemoptysis, night sweats, chills and intermittent low fever. Roentgenologic examination of the chest shows miliary mottling throughout both lungs in almost all cases. Most of the patients recover after prolonged illness. Although extensive clinical studies have been carried out, and in 1 case autopsy,[1] uncertainty still prevails as to the genesis of this disease.

In the opinion of Sodeman and Pullen [2] the etiologic agent and the mechanism of the changes are obscure, while Jamison and Hopkins [3] have expressed the belief that micro-organisms, probably fungi, growing on and causing deterioration of the fibers, and inhaled with these, cause the disease. Castleden and Hamilton-Paterson,[4] on the basis of positive cutaneous reactions obtained with extracts of bagasse, interpreted the disease as an allergic reaction. LeMone, Scott, Moore and Koven [5] in a recent report attributed the changes to the high silica content of the bagasse fiber.

1. Sodeman, W. A., and Pullen, R. L.: New Orleans M. & S. J. 95:558,1943.
2. Sodeman, W. A., and Pullen, R. L.: Arch. Int. Med. 73:365,1944.
3. Jamison, C., and Hopkins, J.: New Orleans M. & S. J. 93:580,1941.
4. Castleden, L. I. M., and Hamilton-Paterson, J. L.: Brit. M. J. 2:478,1942.
5. LeMone, D. V.; Scott, W. G.; Moore, S. and Koven, A. L.: Read at the Thirty-Second Annual Meeting of the Radiological Society of North America, December 1946.

Attempts to reproduce the disease in animals were reported as unsuccessful.[2]

The present investigation was undertaken to determine experimentally the pathogenic properties of bagasse fibers and of the microbial contents of representative suspensions. The possibility that other factors, such as hypersensitivity, play a role in the development of the experimental disease was also explored.

<div style="text-align:center">MATERIAL AND METHODS</div>

Five lots of bagasse were obtained by courtesy of Dr. W. H. Reinhart, of the Louisiana State Department of Health. These were arbitrarily called A, B, C, D and E. Lots A and E were employed for these experiments. Lot A had been baled in January 1944 and stacked for two years. Lot E was a sample of dust collected from rafters near the conveyer in a bailing shed.

For experimental intravenous injections, bagasse was ground in a Waring blender, sterile saline solution added and the suspension filtered through sterile cheesecloth. The suspended particles varied in size from less than 1 micron to 15 microns. They were counted in a hemocytometer, and the suspension was adjusted so that 7,500 particles were present in a cubic millimeter. For intratracheal application, suspensions containing 30,000 to 35,000 particles per cubic millimeter of a size up to 100 microns were made up.

Rabbits and guinea pigs of similar weights and ages were employed in the experiments.

<div style="text-align:center">RESULTS</div>

Intravenous Administration.—Rabbits receiving intravenous injections of a suspension of bagasse A (5 doses of 5 cc. each at three day intervals) rapidly became sick, listless and dyspneic and showed paralysis of the hindlegs. One of them died on the third day, and the three remaining animals were so sick that they were killed on the fourth day. When, however, an aliquot of the bagasse suspension was autoclaved (twenty minutes at 15 pounds [6.8 kg.] of pressure) similar amounts could be given without any apparent ill effect. For control purposes, 2 animals given the autoclaved suspension were killed at an interval corresponding to that of deaths in the first group. The lungs and parenchymatous organs of the animals given the fresh bagasse suspension showed extensive foci of necrosis and marked cellular reaction (fig. 1), indicating that this suspension contained a powerful pathogenic agent. The presence of mycelia and spores (fig. 2) in the organs showing the severest damage served as a clue to the nature of the agent. It is noteworthy that similar lesions could be produced by a single injection of a more concentrated suspension.

In contrast, preparations representing the rabbits given injections of the autoclaved bagasse suspension revealed only small granulomatous lesions of the foreign body type in the lung (fig. 3). Necrosis was absent, and the other organs were free of lesions.

It was then established that autoclaved bagasse suspensions could be given repeatedly without any adverse effect. A rabbit that received 16 intravenous injections, totaling 74.5 cc., over a period of thirty-nine days was killed thirty-eight days after the last injection. Lesions were not seen grossly or microscopically except for an occasional small group of monocytes containing small, irregularly shaped foreign bodies, noted in sections of the lung.

These results suggested that one or several types of micro-organisms present in the bagasse were the pathogens, while the fiber itself under the conditions tested produced only a foreign body reaction of no apparent consequence to the experimental animal.

In the bacteriologic studies five different micro-organisms were isolated from bagasse A suspension and cultured on various mediums under aerobic conditions: Organism 1 gave the reactions of the group of organisms designated bacteriologically as Bacillus polymyxa. Organism 2 was a characteristic form of Aspergillus fumigatus. Organism 3 was an aerobic member of the Actinomyces group. Organism 4, while differing in gross appearance from 2, showed microscopic characteristics that place it in the Aspergillus fumigatus group also. Organism 5 was an aerobic motile spore-formig gram-positive rod which failed to liquefy gelatin and had many, though not all, of the characteristics of the Bacillus circulans group.

Similar results were obtained with the other lots of bagasse except that organism 4 could not be isolated from lot[1] B.

Suspensions were prepared by washing the agar slants with saline solution. They were standardized bacteriologically to contain approximately 10,000 organisms per cubic millimeter. The elements of the Aspergillus and Actinomyces cultures were similarly counted by attempting, as far as possible, to estimate the number of particles—mycelia or spores—present. It was planned to inject 5 cc. of one or another of the suspension intravenously into each rabbit twice. Suspensions of 1, 3, 4 and 5 were well tolerated. Rabbits, however, which had received a single injection (5 cc.) of suspension 2 (aspergillus) invariably died or were so sick two to three days after the first injection that it was preferred to put them to death.

The lungs, hearts, livers and kidneys of these rabbits showed numerous lesions of a necrotizing character. Mycelia were sometimes histologically demonstrable, and A. fumigatus was recovered from a blood culture at autopsy.

Sections representing the rabbits inoculated with the other micro-organisms and killed at similar intervals revealed that organisms 1 and 3 produced only minimal foreign body reaction in the lung. The cellular reaction of the lungs of the animals inoculated with organisms 4 and 5 was also slight, but abscess formation was observed in the liver in both instances. Numerous multinucleated giant cells were noted within the granulation tissue. Structures phagocytosed in their cytoplasm suggested that the giant cells were macrophages which had ingested some of the micro-organisms.

Three rabbits that were given suspensions of bagasse E in amounts and over periods similar to those employed in the first two groups withstood the treatment well. Two of them, on histologic study, showed minimal pulmonary lesions, one of them also a renal abscess, while the lungs of the third animal showed numerous foci of mononuclear exudate and of giant cell formation; polymorphonuclear leukocytes were rare, and necrosis was absent.

Intratracheal Administration.—The fact that bagasse injected intravenously produced severe lesions did not necessarily imply that it would call forth a similar reaction when introduced by way of the respiratory tract. A group of rabbits received, therefore, bagasse suspensions by intratracheal insufflation. For this the animals were under combined local and light ether anesthesia.

Each rabbit was given 10 cc. of suspension, an amount of fluid that is absorbed from the lungs within seventy-two hours.[6] For control purposes aliquots of the

---

6. Courtice, F. C., and Phipps, P. J.: J. Physiol. **105**:186, 1946.

suspensions were either autoclaved or formaldehydized by addition of 10 per cent (volume) of a 40 per cent solution of formaldehyde. After three to four days the formaldehyde solution was removed by centrifuging the particles and resuspending them in saline solution. These suspensions were sterile on culture. The animals were killed after ten days. Microscopic sections of the lungs of rabbits treated with the suspension of fresh bagasse showed numerous large pneumonic foci (fig 4). The exudate was composed of polymorphonuclear leukocytes, monocytes and multinucleated giant cells, with frequent disintegration of the exudate cells. The giant cells were of the foreign body type and contained variously shaped cytoplasmic defects which under polarized light sometimes corresponded to double-refractile, apparently unstained foreign bodies. The interstitium was infiltrated by round cells. There was fibroblastic proliferation at the periphery of the lesion. Some bronchial lumens contained desquamated epithelial and disintegrated exudate cells. An occasional vessel was occluded by granulation tissue and its wall infiltrated by small round cells (fig. 5). One liver revealed numerous small foci of necrosis and infiltrating polymorphonuclear leukocytes.

In sections of the lungs of the control animals, lesions were infrequent, small and of granulomatous character. Giant cells were fairly numerous, but polymorphonuclear leukocytes were encountered only occasionally, and cellular disintegration was absent (fig. 6). Fibroblastic proliferation was more intense than in the first group. The vessels were free of lesions.

Suspensions of bagasse E were administered to 2 rabbits. After ten days their lungs contained fairly frequent foci of pneumonic exudate, with monocytes being prevalent in the larger and polymorphonuclear leukocytes in the smaller lesions. Multinucleated giant cells were also present. Cellular disintegration and interstitial infiltration were, however, less prominent than in the group which had received bagasse A.

Guinea pigs were subjected to similar procedures, with suspension of bagasse E, except that the amount insufflated was 3 cc. Sections of the lungs of 2 animals obtained at a ten day interval revealed several foci of a polymorphonuclear and monocytic alveolar exudate. Several alveoli in these groups contained multinucleated giant cells, one to each. Sporelike foreign bodies were seen in the cytoplasm of these cells. There was no necrosis, and no changes were found in the other organs.

Intracutaneous Tests.—It has been stated earlier that intracutaneous tests carried out with extracts of bagasse by two groups of authors yielded different results. This problem was therefore also investigated. Rabbits which had received two intravenous injections each of 5 cc. of bagasse suspension over a period of six days were tested by intracutaneous injection of 0.05 cc. of Seitz filtrates prepared from saline suspensions of cultures of each of the five micro-organisms mentioned earlier. The tests were made thirteen days after the first injection of the bagasse suspension.

With filtrates 1 and 4 there was a reddening of the skin within a few hours after the injection, and the reaction increased in intensity until about twenty-four hours later. A slight reaction was observed in one instance with filtrate 2. The other filtrates and saline solution, which was used as control, gave negative results. A normal rabbit, however, tested with identical preparations showed similar results.

That sensitization is not an essential factor in the genesis of the experimental disease was also indicated by the fact that a single injection of a more concentrated suspension of bagasse A produced lesions similar to those due to repeated injections.
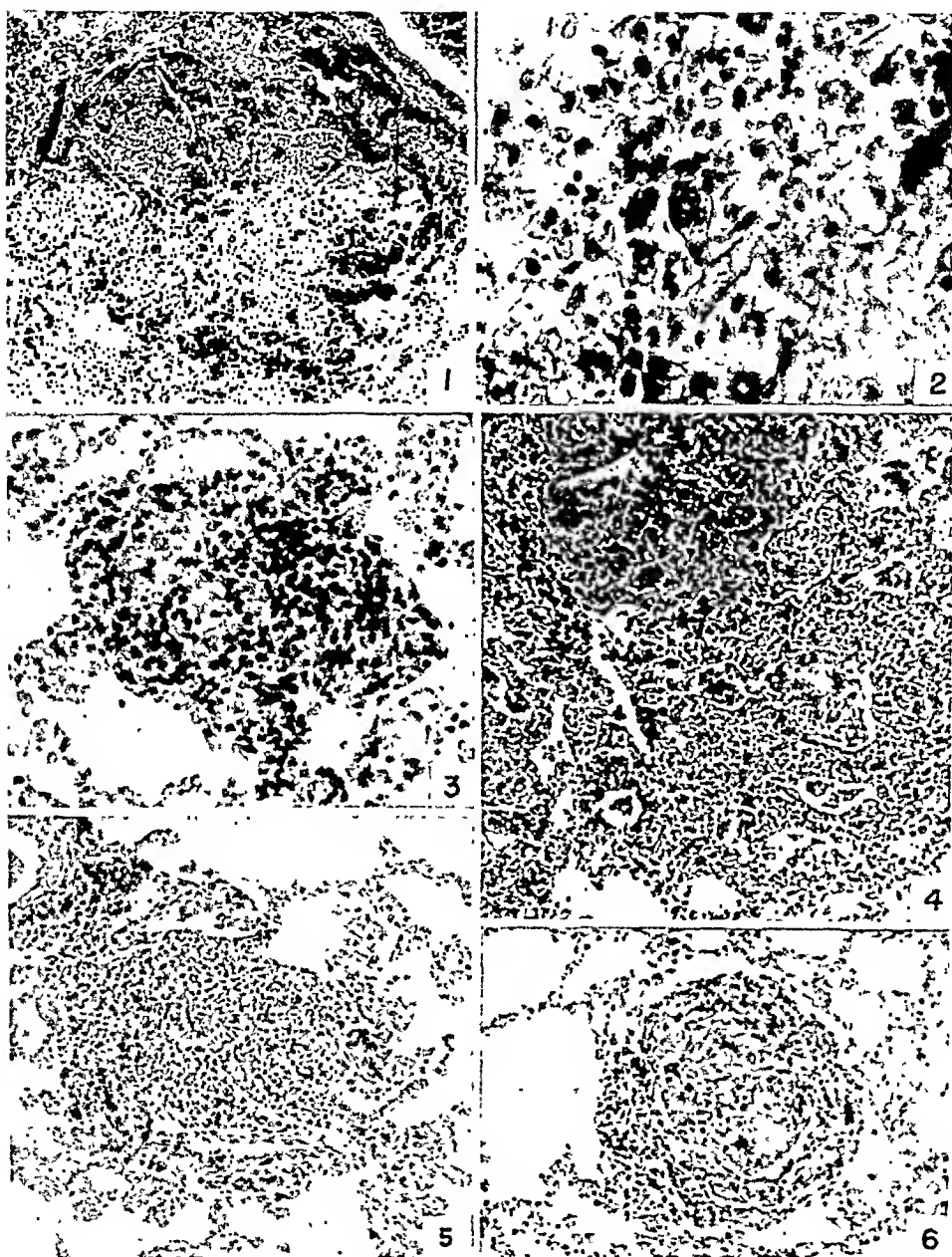
Fig. 1.—Rabbit given intravenous injections of a suspension of unheated bagasse A; third day; lung; focus of necrotizing pneumonia; Masson stain; × 75.

Fig. 2.—Rabbit given intravenous injections of a suspension of unheated bagasse A; third day; kidney; mycelial fragment and spore; Masson stain; × 476.

Fig. 3.—Rabbit given intravenous injections of a suspension of autoclaved bagasse A; third day; lung; granulomatous lesion; hematoxylin and eosin; × 238.

Fig. 4.—Rabbit given intratracheal insufflation of fresh bagasse; tenth day; lung; pneumonic exudate of polymorphonuclear leukocytes, monocytes and foreign body giant cells; hematoxylin and eosin; × 136.

Fig. 5.—Rabbit given intratracheal insufflation of fresh bagasse; tenth day; lung; vessel occluded by granulation tissue: hematoxylin and eosin × 156.5.

Fig. 6.—Rabbit given intratracheal insufflation of autoclaved bagasse; tenth day; lung; foreign body granuloma; hematoxylin and eosin; × 177.

COMMENT

In the cases reported so far human bagasse disease occurred after an exposure of several weeks or months. The pathologic changes described here resulted from short term experiments and should not be taken, therefore, as a basis for comparing the lesions morphologically.

Suspensions of unheated bagasse given intravenously proved fatal to rabbits except when given in small doses. The fact that numerous necrotizing lesions with fragments of fungi demonstrable within them were found in the kidney and the liver while the highly refractory bagasse fibers were not seen there, together with the fact that there were no lesions in these organs in animals given injections of autoclaved bagasse, suggests that the fiber itself does not contribute to the causation of the experimental disease. It may be assumed, however, that the process of autoclaving changed the structure of the fiber, rendering it less harmful. Thus, resort was taken to formaldehydizing, a procedure that would affect the micro-organisms of the suspension and change only amino groups of protein particles possibly present in the bagasse fibers. The pathogenicity of bagasse, however, was equally abolished by the second procedure.

Among the micro-organisms isolated from bagasse, the aspergilli proved to be the most harmful for rabbits. These are ubiquitous fungi and are known not only for their toxic effect when injected intravenously into animals but also for their occasional pathogenicity for man. In view of the cosmopolitan distribution of A. fumigatus in the soil and in vegetable matter, it is difficult to attribute human bagasse disease to these organisms alone, since similar respiratory disease has not as yet been noted among many other occupational groups equally exposed to these fungi. Experiments employing prolonged exposure or providing longer intervals are necessary to decide whether aspergillosis, alone or in combination with other factors, simulates in its morphologic aspects human bagasse disease.

A slight but distinct difference was noted between the lesion produced by bagasse A and that by bagasse E, the changes produced by the latter being less severe. Bagasse disease has, however, rarely if ever occurred in men working in places where bagasse E was collected, and thus its pathogenicity for rabbits would represent a failure to supply the experimental corollary. Among the microbial contents of bagasse E, however, were also aspergilli, and it may well be that the particular susceptibility to these fungi enhanced the severity of the lesions in rabbits. The less serious character of the lesions occurring in guinea pigs would support this assumption.

SUMMARY

Final interpretation of the results of these experiments has to be postponed until more information concerning the pathogenicity of the micro-organisms and of the fibers described will be available. Preliminarily it may be said, however, that the bagasse fiber itself under the test conditions described is a rather inert material, that the acute inflammatory process caused by bagasse is due to micro-organisms, probably fungi, attached to the bagasse, and that the lesions in no instance resembled those in human or experimental silicosis.

RHABDOMYOSARCOMA OF THE BLADDER
Report of a Case and Review of the Literature

HERMAN L. KRETSCHMER, M.D.
CHICAGO

NONEPITHELIAL tumors of the urinary bladder are of infrequent occurrence, and sarcoma is the most rare. The rarity of sarcoma of the bladder is evidenced by its low incidence in reported collections of tumors of the bladder. Albarran [1] observed 2 sarcomas among 89 vesical neoplasms; Caulk,[2] 1 in 303; Davis,[3] 2 in 41; Egger,[4] 1 in 83; von Frisch,[5] 1 in 300; Scholl,[6] 1 in 262; Ratliff and Valk,[7] 3 in 552; Wheelcock [8] encountered 3 in 58,437 surgical specimens and 2,783 autopsies at the Deaconess Hospital, Boston, and at Pondville Hospital, Wrentham, Mass., only 1 sarcoma was found among 288 primary vesical tumors; Pack and LeFevre [9] in a survey of neoplastic diseases at the Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York, from 1917 to 1929 found only 2 cases of sarcoma of the bladder among 19,129 cases of tumor. Crane and Tremblay [10] reviewed the literature in 1943 and were able to collect only 151 cases of primary vesical sarcoma.

Many histologic types have been reported: angiosarcoma, leiomyosarcoma, fibromyxosarcoma, neurogenic sarcoma, fibrosarcoma, rhabdomyosarcoma, rhabdomyomyxosarcoma and osteogenic sarcoma. No detailed discussion of sarcoma in general will be undertaken, as several excellent reviews have appeared in the literature. Apart from chondrosarcoma and osteogenic sarcoma, rhabdomyosarcoma and rhabdomyomyxosarcoma are among the rarest tumors to be found in the bladder.

1. Albarran, J.: Ann. d. mal. d. org. génito-urin. 15:785,. 1897.
2. Caulk, J. R.: J. Urol. 16:211, 1926.
3. Davis, L.: Ann. Surg. 43:566, 1906.
4. Egger, O.: Ztschr. f. urol. Chir. 6:175, 1921.
5. von Frisch, A.: Wien. klin. Wchnschr. 20:1205, 1907.
6. Scholl, A. V.: Surg., Gynec. & Obst. 34:189, 1922.
7. Ratliff, R. K., and Valk, W. L.: J. Urol. 42:559, 1939.
8. Wheelcock, M. C.: J. Urol. 48:628, 1942.
9. Pack, G. T., and LeFevre, R. G.: J. Cancer Research 14:167, 1930.
10. Crane, A. R., and Tremblay, R. G.: Ann. Surg. 118:887, 1943.

To date there have been reported 16 cases of rhabdomyosarcoma and rhabdomyomyxosarcoma, and my own case is the seventeenth to be recorded. Only 15 cases of those recorded in the literature have been summarized in the table, as the article by Janu and Stolz [11] was not available.

REPORT OF CASE

Mr. G. J. H., aged 53, entered the Presbyterian Hospital, Chicago, July 13, 1942. He was married and had two children, living and well. At the age of 35 he suffered an attack of gonorrhea, from which he made a complete recovery. The family history was noncontributory. Two weeks prior to hospitalization profuse hematuria developed. The day following the onset of the hematuria frequency of urination developed and continued both day and night. Some burning and urgency of urination were noted.

Examination showed head, neck, heart, lungs and abdomen normal. There was a small hydrocele on the the right side. Rectal examination revealed no abnormality. The right lower extremity showed marked varicose veins. The Kahn test was negative. Roentgen films, including intravenous pyelograms, showed no evidence of disease. The blood count showed 12,300 leukocytes per cubic millimeter; otherwise the blood was normal. The urinalysis showed albumin (1 plus); the urine contained no sugar, and the sediment showed red blood cells. All cultures of the urine were sterile except the ascitic fluid broth, which showed a scant growth of small gram-negative cocci after four days.

Cystoscopic examination showed a tumor, the size of a large walnut, in the dome of the bladder.

Suprapubic cystotomy was done on July 21, with resection of the tumor, including the involved wall of the bladder and fulguration of the area of excision. The patient made an uneventful recovery and was discharged August 5.

He applied for insurance in May 1946. The report from the insurance company, dated May 29, 1946, stated that physical examination gave negative results, that the urinalysis revealed no abnormality and that the patient had no symptoms.

On September 26, cystoscopic examination showed no abnormality, and there was no sign of tumor.

The pathologic diagnosis, made by Dr. George M. Hass, of the Presbyterian Hospital, was: rhabdomyosarcoma of the urinary bladder. His report follows:

"There are numerous mitotic figures, and the cells are poorly differentiated. It is difficult to classify the tumor, which is not the usual type of fibrosarcoma. The cells are in keeping structurally with cells of leiomyosarcoma or those of rhabdomyosarcoma. This means that the tumor has cells in it that are very similar to those that are found in rhabdomyosarcoma of the heart, the skeletal muscle or mixed tumors, especially those of the kidney. Inasmuch as tumors of this type, especially mixed tumors, have been frequently found in relation to the urinary tract and occasionally in relation to the bladder, I am inclined to classify this tumor as rhabdomyosarcoma."

---

11. Janu, M., and Stolz, J.: Časop. lék. česk, **71**:15, 1932. (Original article not available for perusal.)

Recorded Cases of Rhabdomyoma and Rhabdomyonyxosarcoma

Author	Sex	Age	History	Result	Location of Tumor	Type of Tumor
Mönckeberg, J. G.: Virchow's Arch. f. path. Anat. 187: 471, 1907	F	23	Hematuria. Removal of tumor. Recurrence. Second removal	Death	Posterior wall of bladder	Rhabdomyosarcoma
MacKenzie, D. W., and Chase, W. H.: J. Urol. 19: 315, 1928	F	69	Hematuria; pain in left loin, radiating to bladder; nausea and vomiting; loss of weight and strength. Readmission for nephritis. Operation for recurrence of symptoms. Cystoscopy with diagnosis of cancer of bladder. Suprapuble cystotomy	Death	Floor of bladder and entire trigone	Rhabdomyosarcoma of bladder extending directly into left ureter, with metastases in duodenum, portal lymph nodes and liver
Houetto [12]	M	13 mo.	Urinary difficulties since birth; mass in pelvic region; uremia	Death	In a congenital diverticulum of bladder	Rhabdomyosarcoma
White, H. P. W.: Proc. Roy. Soc. Med. 22: 1382, 1929	Not stated	1 yr. 10 mo.	Swelling in lower part of abdomen; urine turbid with pus. Cystoscopy and cystogram led to diagnosis of tumor of bladder. Excision of tumor with partial resection of bladder	Result not stated, but prognosis poor	Anterior wall of bladder	Rhabdomyosarcoma
Monserrat, J. L., and Garcia, A. E.: Hosp. argent. 3: 856, 1933	M	43	As original article was not available for perusal, data on history cannot be supplied; data given here have been obtained from other authors	Death	Right wall of bladder	Rbabdomyosarcoma
Wolfold, J.; Hill, L., and Hillebrand, J. G.: J. Urol. 36: 150, 1936	F	Infant	Incontinence, abdominal pain, hematuria. Cystogram. Diagnosis: vesical tumors. Cystotomy. Microscopic examination made of some pieces of tumor. No treatment	Death	Not stated	Rhabdomyomyxsarcoma
Idem	M	2	Straining on urination, frequency; later, incontinence; paroxysmal pains, Exploratory cystostomy. Removal of tumor of bladder. Third day after operation tumor tissue noticed fungating through bladder opening. Roentgen irradiation, application of radium and excision of mass	Death	Anterior wall of bladder	Rhabdomyomyxsarcoma
Vermooten, V.: J. Urol. 42: 126, 1939	F	20 mo.	For 6 months difficulty in passing urine; blood in urine; constipation. Catheterization for retention of urine	Death	Trigone	Rhabdomyosarcoma
Ratliff and Valk [7]	M	6	Nocturia, frequency, straining and dysuria for 6 mo., terminating in acute retention 3 days prior to admission. Suprapuble inspection showed neoplastic prostate and complete infiltration of vesical neck. Tumor stripped from base, and base cauterized. Radium used 25th day following removal of recurrent tumor; 5 mo. later, tumor completely filled bladder; enucleation; 2 mo. later, excision; 1 mo. later, recurrence	Death	Entire bladder	Rhabdomyomyxsarcoma
Idem	M	65	Progressive intermittent hematuria for 8 mo.; frequency, straining and mild tenderness over bladder area. No treatment	Death	Tumor attached to pedicle 2 cm. above and to left of left ureteral orifice	Rhabdomyosarcoma

Reference	Sex	Age	Clinical history	Outcome	Location	Diagnosis
Uhlmann, E.; Grossman, A., and Calvin, J. K.: Ann. Surg. 109:624, 1939	M	10 mo.	Pain on urination. Suprapubic cystotomy, 1 week later fungating mass had grown out to anterior abdominal wall at site of cystotomy. Radiation therapy	Death	Tumor arose from outer coats of wall of bladder; the space of Retzius completely filled by tumor; mucosa apparently not involved	Rhabdomyosarcoma
Hunt[13]	M	Newborn Infant	72 hr. after birth cyanosis of legs and abdominal distention; painful mass in suprapubic area. Catheterization for acute retention; development of urinary fistula. 65 days after birth, cystostomy; nodular mass covering floor of bladder with greater concentration around neck of bladder; biopsy showed rhabdomyoma, 2d biopsy showed rhabdomyosarcoma. 172 days after birth, total cystectomy, prostatectomy, transplantation of ureters. Later, transverse colostomy and right ureterosigmoid anastomosis	Death	Floor of bladder; greater concentration around bladder neck	Rhabdomyosarcoma
Idem	M	2½	Pain on urination and retention of urine. Cystoscopy showed tumor of bladder. Suprapubic cystostomy with fulguration of tumor; roentgen therapy. Rapid recurrence of tumor; hydronephrosis. Right nephrostomy for drainage, transfusions, total cystectomy and prostatectomy, with transplantation of left ureter. Several subsequent recurrences; mass eventually closed off ureter	Death	Entire bladder	Rhabdomyosarcoma
Khoury and Speer[14]	M	Infant	2 mo. after birth, cystotomy for umbilical fistula; nodular mass found in bladder; later, diarrhea, vomiting, pyrexia, and voiding of urine through suprapubic wound; these symptoms lasted 11 mo. Total cystectomy and abdominal ureterostomy followed by 2 stage bilateral ureterosigmoidostomy. Growth had caused hydronephrosis, ascending urinary infection and urachal fistula	Death	Trigone; growth surrounded the urethra and infiltrated wall of bladder, prostate and verumontanum	Rhabdomyosarcoma
Hirsch and Gasser[15]	M	5	Frequency, urgency, voiding small quantities of urine; distended bladder; rectal tumor. Suprapubic cystotomy; tissues removed for histologic examination. Suprapubic cystotomy. Roentgen therapy	Death	Extramural, in the tissues behind trigone and prostatic urethra	Rhabdomyosarcoma
Kretschmer, H. L.: Arch. Path., this issue	M	53	Hematuria, frequency, burning and urgency of urination. Cystoscopic examination: vesical tumor. Suprapubic cystotomy; resection of tumor, including wall of bladder; fulguration of area of excision	Recovery. Cystoscopic ex. 4 yr. 2 mo. after operation showed bladder normal	Dome of bladder	Rhabdomyosarcoma

353

A specimen was sent to the Army Medical Museum, and Dr. Raymond O. Dart, who at present is in charge of the Tumor Registry of the American Urological Associations, reported as follows:

" . . . it has been found expedient to use the generic term 'myosarcoma' in many cases. The reason for this is that the cellular composition of many of the prostatic and vesical tumors of muscle varies from undifferentiated elements all the way to frankly striated cells, often varying during the progression of the disease. The present tumor contains some large acidophilic elements, and it is highly probable that it is rhabdomyosarcoma."

COMMENT

Rhabdomyosarcoma is the cancerous variant of rhabdomyoma, and both types have their origin in striated muscle fibers. Rhabdomyoma and rhabdomyosarcoma have been reported as occurring in the heart, the skeletal musculature, the testicles, and the vagina, but the bladder has seldom been the seat of either tumor. However, one can only speculate as to the embryogenesis of these rare tumors, and there is no unanimity of opinion.

Wilms [11a] and Houette [12] expressed the belief that each of these tumors arises from embryonal heterotopia due to an anomaly of the development of the wolffian canal. Shattock was inclined to think that rhabdomyoma arises from vagrant sarcoblasts which have abnormally extended or have been displaced beyond their usual deeper limits into the subepithelial tissue of the bladder. Hunt [13] stated that although one can never prove the origin of these tumors, it is probable that they arise from the striated muscle elements normally present in the anterior part of the prostate and in the region of the internal sphincter and adjacent area of the trigone. Khoury and Speer [14] also expressed the opinion that it is plausible that the neoplasm containing striated tissue could arise from the striated muscle elements normally present in the sphincter of Henle, but this theory does not explain how these tumors can arise at a distance from the neck of the bladder. They also called attention to the fact that rhabdomyosarcoma of the prostate may be confused with rhabdomyosarcoma of the bladder.

Hirsch and Gasser [15] reported an extramural rhabdomyosarcoma of the urinary bladder that occurred in the tissue behind the trigone and the prostatic urethra and mentioned that extramural rhabdomyosarcoma has been reported as primary in the prostate; they are inclined

11a. Wilms, M.: Die Mischgeschwülste, Leipzig, Arthur George, 1899

12. Houette, C.: Ann. d'anat. path. **6:**267, 1929.

13. Hunt, R. W.: New York State J. Med. **43:**513, 1943.

14. Khoury, E. N., and Speer, F. D.: J. Urol. **51:**505, 1944.

15. Hirsch, E. F., and Gasser, G. W.: J. Urol. **51:**517, 1944.

to favor the conclusion that these tumors are primary in the prostate or that they may belong with rhabdomyosarcoma occurring along the vas deferens. They stated,

. . . The hypoplasia (or atrophy) of the right vas deferens observed with the rhabdomyosarcoma of this report suggests that there have been disturbances in growth or development of tissue about the spermatic cord in the region of the prostate. The rhabdomyosarcoma cell invasion of the puerile prostate and seminal vesicle tissues, which is comparable to the cell invasion of the wall of the urinary bladder, leaves the impression also that both the prostate and the urinary bladder have been invaded secondarily.

Since there is confusion as to the origin of rhabdomyosarcoma of the bladder and it has been suggested that it may start from the prostate, I wish to call attention to the fact that the tumor in my case was located in the dome of the bladder. This excludes the possibility that it took its origin in the prostate, and excludes also the possibility that it took its origin in the vas deferens or the seminal vesicles, invading the bladder secondarily, as has been stated by several of the previously mentioned authors.

PROGNOSIS

An analysis of the cases reported shows that the prognosis is poor in patients having this highly malignant and rapidly recurring tumor. All of the cases resulted fatally. In the case reported here there has been no recurrence to date and the patient is free of symptoms four years and two months after operation.

SUMMARY

A review of the literature and my own case brings the number of reported cases of rhabdomyosarcoma of the urinary bladder to 16. Eleven of the patients were under 10 years of age.

LIMITATION OF SPREADING FACTOR IN INJURED RATS

ISOLDE T. ZECKWER, M.D.

PHILADELPHIA

IT IS NOW well established that after many injuries of the body the adrenal cortex, by a reaction mediated through the anterior lobe of the pituitary gland, promptly discharges a hormone which destroys lymphocytes, thereby releasing antibodies and causing shrinkage of lymphatic tissue. This constitutes a coordination of defense reactions when the injury is a bacterial agent. However, this chain of reactions occurs, ending in destruction of lymphocytes, whether the injurious agent is a bacterial or whether it is a physical or a chemical one. Presumably, the lymphocyte when disrupted may release other protective substances besides antibodies.

Since under many pathologic conditions in which bacteria are not present, such as healing of bland infarcts, degeneration of goiters and healing of sterile wounds, lymphocytes occur in tissue simultaneously with fibroblasts, it has been thought that the lymphocytes might make possible the laying down of collagen by connective tissue cells. In bacterial infections, it is now well known, the spread of the inflammatory process is greatly enhanced by hyaluronidase, which hydrolyzes the hyaluronic acid in the intercellular substance formed by connective tissue. If in sterile areas of destruction of tissue hyaluronidase should be present, it would seem possible that the healing might be aided by lymphocytes releasing a substance that limits the action of hyaluronidase and thus creates the conditions which permit connective tissue cells to lay down collagen. If this were so, it would give new meaning to the adrenocortical-lymphatic tissue reaction to injury.

Certain observations reported in the literature suggest that the spread of hyaluronidase may be influenced by pituitary-adrenocortical activity. It has been shown by Weinstein[1] that an anterior pituitary extract when injected repeatedly into rabbits caused limitation of the spread of testicular extract injected intracutaneously. Cahen and Granier[2] observed that spread of hyaluronidase is restricted in animals given previous injections of morphine. Epinephrine, also, caused restriction of the spreading fac-

---

1. Weinstein, L.: Yale J. Biol. & Med. **12**:549, 1940.
2. Cahen, R. L., and Granier, M.: Yale J. Biol. & Med. **16**:257, 1944.

tor, according to investigators cited by Cahen and Granier.[2] Both morphine and epinephrine were listed by Selye [3] as damaging agents which incite the "alarm reaction" resulting in shrinkage of lymphatic tissue which is now known to be caused by pituitary-adrenocortical activity. Morphine is known to stimulate the adrenal medulla to secrete epinephrine, and epinephrine has been shown by Vogt [4] and by Long and Fry [5] to cause secretion on the part of the adrenal cortex.

In view of these data and in view of the important part played by hyaluronidase in enabling bacteria to spread through connective tissue, it was thought of interest to see whether limitation of spread occurred during the pituitary-adrenocortical-lymphatic tissue reaction which occurs as a result of various injuries of the body (bacterial, chemical or physical).

With this idea in mind, it was determined to study quantitatively the spread of ink combined with a spreading factor in the skin of the abdomen of rats subjected to injury of the leg muscles and to compare this with the spread in uninjured rats. The dose and intervals of time used were those which had proved effective in activating the pituitary-adrenocortical dissolution of lymphocytes in previous experiments.[6]

EXPERIMENTAL PROCEDURE

Fourteen pairs of rats were used, each pair of the same sex and either litter mates or of the same weight. One of each pair received intramuscularly 0.5 cc. of solution of formaldehyde U. S. P. diluted 1:25 in each of the four legs, one at a time, over a period of two days. In a few rats trypsin solution was injected instead of solution of formaldehyde, to cause injury. The control either received no injection or received injections of isotonic solution of sodium chloride. At intervals varying from two to nineteen hours after the last injection, the rats were anesthetized with ether, the hair was removed from the chest and the abdomen by electric clippers, and intracutaneous tests were made for spread. The spreading factor used was, as a matter of convenience, derived from rat testes rather than from bacteria. The testes were ground first in water; then an equal volume of 1.8 per cent sodium chloride solution was added, and the mixture was centrifuged. As many cubic centimeters of solution were added as there were grams of tissue. This preparation was kept in the refrigerator, during which time it increased in potency; it was subsequently diluted with saline solution for a convenient degree of spread. The supernatant liquid was drawn up to 0.15 cc. in a tuberculin syringe, then 0.05 cc. of india ink (not waterproof) suspension which had been diluted previously 1:2 with 0.9 per cent salt solution was drawn into the syringe, and the mixture was injected into the skin of the abdomen. A control injection of 0.15 cc. of a 0.9 per cent sodium chloride solution with 0.05 cc. ink solution was made in the skin of the thorax. When the formaldehyde solution had been injected into the rat, a great

3. Selye, H.: Endocrinology **21**:165, 1937.

4. Vogt, M.: J. Physiol. **103**:317, 1944.

5. Long, C. N. H., and Fry, E. G.: Proc. Soc. Exper. Biol. & Med. **59**:67, 1945.

6. Zeckwer, I. T.: Proc. Soc. Exper. Biol. & Med. **60**:66, 1943

deal of edema occurred at the site and sometimes extended toward the abdomen. The ink was injected as far as possible from the edematous area.

The spread of the dye was traced on cellophane at appropriate intervals. One and two hours after the injection of ink were found to be convenient times for reading the spread. The area was measured by a planimeter in square inches

Experimental Observations

Experimental Group	Mean Spread of Ink		Mean Percentage Reduction of Spread in Injured Rats as Compared with Controls
	In Control Rats	In Injured Rats	
14 pairs of intact rats..............	2.01 sq. in. (13 sq. cm.)	1.12 sq. in. (7 sq. cm.)	44%
4 pairs of adrenal-ectomized rats..	2.60 sq. in. (16.5 sq. cm.)	1.16 sq. in. (7.5 sq. cm.)	58%
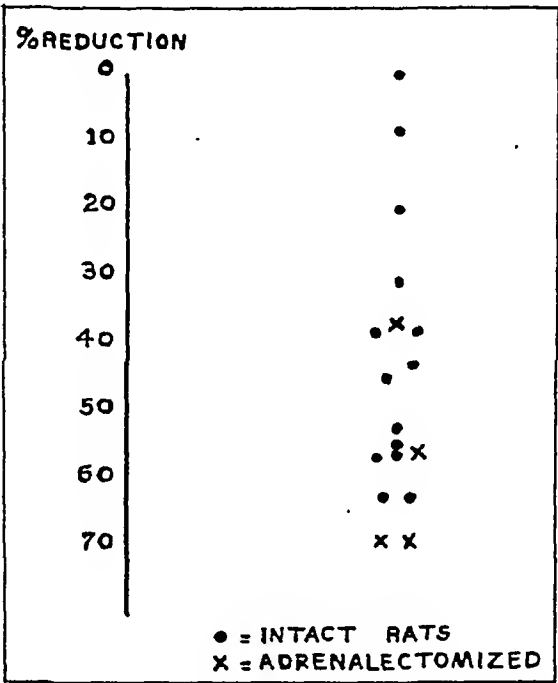
Percentage reduction of the spread of a mixture of ink and testicular extract in injured rats as compared with uninjured rats.

(table), and the amount of spread observed in the injured rat was calculated as a percentage change of that observed in the control rat of each pair (figure). As different concentrations of hyaluronidase were used as spreading factors from day to day, comparisons cannot be made between all of the injured rats and all of the controls. On the contrary, each test rat should be compared with its own control which received the same spreading factor.

It was soon obvious that injured rats showed marked limitation of the spread. It then became necessary to see whether this depended on adrenocortical activity. The experiments were repeated with four pairs of adrenalectomized rats. Adrenalectomy was performed through a single midline incision of skin carried down the back, with incision through muscles and peritoneum on each side of the vertebral

column, so as to avoid any traumatizing of the abdominal area. The rats were maintained in good condition on saline drinking solution for one to three weeks before the spread was determined. The tests for spread were made two to five hours after the last injection of formaldehyde solution. The adequacy of the removal of functioning adrenocortical tissue was evidenced by (1) the fact that some animals died during the day following the last injection of formaldehyde solution, or (2) the fact that there was hypertrophy of lymphatic tissue with failure to shrink after injections of formaldehyde solution in those rats which were killed after completion of the experiment, or (3) the fact that spontaneous death of the rat occurred when salt was withdrawn from the drinking water. As will be seen from the chart, the injured adrenalectomized rats showed just as much limitation of the spread of ink as did the intact injured rats. Hence, responsibility of the adrenocortical-lymphatic tissue reaction can be ruled out.

COMMENT

Since the restriction of spread does· not depend on adrenocortical activity, the only other obvious possibility is that the edema of the legs following injury might lead to relative dehydration of the abdominal area given injections of dye and that this dehydration might limit the spread through connective tissue. Duran-Reynals [7] stated that when testicular extract is injected directly into an edematous area the spread is restricted. No data were found on the effect of dehydration. According to Duran-Reynals [7]: "It would seem that the spreading is a phenomenon in which vasomotor or other nervous reactions play no part." When the spreading factor is injected directly into an area of injury or inflammation, the spread is limited, but in the present experiments the site of injection of the spreading factor was distant from the injured area. Hechter [8] found that ischemia from exsanguination did not influence the spread of hyaluronidase and concluded that the spread "is not influenced by changes in skin blood flow."

SUMMARY

The spread of a mixture of ink and testicular extract (hyaluronidase) injected intracutaneously into the abdomen was restricted in rats whose leg muscles had been injured by injections of formaldehyde solution. This restriction also occurred in adrenalectomized rats, and consequently it did not depend on the adrenocortical-lymphatic tissue reaction to injury.

7. Duran-Reynals, F.: Bact. Rev. **6**:197, 1942.
8. Hechter, O.: J. Exper. Med. **85**:77, 1947.

HISTOLOGIC ASPECTS OF THE SKIN IN THE LATE STAGES OF TRENCH FOOT

MATTHEW BLOCK, M.D.*

CHICAGO

THE PURPOSE of this investigation was to study the pathologic aspects of the chronic form of trench foot, to determine the mechanisms responsible for the accompanying clinical symptoms and thus to lay the groundwork for a more rational therapy. Although from the point of view of pathology a great deal of information about trench foot based on amputation material has been accumulated,[1] there has been little concerning the late sequelae, especially in cases in which there was no loss of tissue. This investigation was based on biopsy specimens taken from 15 patients with uncomplicated trench foot in the chronic state and from 8 controls.

Trench foot occurred in these patients as a result of their being exposed to cold wet weather over periods varying from days to weeks. Other factors resulting from unavoidable circumstances of combat adversely affected the course of the disease. Some of the men were unable to change their wet shoes or socks for as long as a week or two; others were forced to live in the snow without shelter for long periods. Because of the tactical situation, many were kept on their feet during the early stages of the disease, others were forced to lie in cramped positions in foxholes, in melted snow up to their waists for hours and days at a time.

In its most characteristic and severe form, trench foot occurred primarily in combat infantrymen. More specifically, it was predominantly a disease of the lower ranks of the ground combat troops, who bore the brunt of the fighting under conditions conducive to the development of the disease.

Trench foot closely resembles other neurovascular diseases of the extremities caused by exposure to cold, as immersion foot,[2] high altitude

frostbite,[2] pernio [4] and frostbite.[5] There is still a great deal of discussion concerning the interrelationships and possible fundamental identity of all these processes. However, trench foot may be separated from the others on the basis of the history that the patient had been exposed to cold wet weather under the circumstances described.

Although trench foot is as old as war in cold wet weather, the first accurate clinical account of the acute phase was written by Larrey [6] on the basis of his experience during Napoleon's retreat·from Moscow. He described the pale, swollen appearance of the feet, coincident with numbness and paresthesias, and the supervening painful erythematous stage, followed by dry gangrene. In the course of several weeks he observed exfoliation of the mumified gangrenous tissue or, in the more severely injured, demarcation and slough of the mummified tissue. The details of this acute phase of trench foot and related diseases may be found in many recent articles.[7]

CLINICAL SYMPTOMS AT TIME OF BIOPSIES

The biopsies were performed on patients from three to four months after evacuation. In the more severe lesions studied, a demarcation zone had formed, and the gangrenous tissues had shrunk so that spontaneous amputation was obviously imminent. In the less severe lesions the shell of superficial gangrenous tissue had been exfoliated and new skin had already developed to cover the toes. All but 2 men were ambulant, although an attempt was made to keep those whose lesions were more severe in bed, to prevent secondary infection.

1. (a) Friedman, N. B.: Am. J. Path. **21**:387, 1945. (b) Siegmund, H.: Zentralbl. f. Chir. **70**:1558, 1943. (c) Lesser, A.: Ann. Surg. **121**:257, 1945.

2. (a) Blackwood, W.: Brit. J. Surg. **31**:329, 1944. (b) White, J.: New England J. Med. **228**:211, 1943. (c) White, J., and Warren, S.: War Med. **5**:6. 1944.

3. Davis, L.; Scarff, J.; Rogers, M., and Dickinson, M.: Surg., Gynec. & Obst. **77**:561, 1943.

4. McGovern, T.; Wright, I., and Kruger, E.: Am. Heart J. **22**:583, 1941.

5. Ducuing, J.; D'Harcourt, J.; Folch, A., and Bofil, S.: J. de chir. **55**:385, 1940.

6. Larrey, D. J.: Mémoires de chirurgie militaire et campagnes, Paris, C. J. Smith, 1812, vol. 3, p. 60.

7. (a) Patterson, R. H., and Anderson, F. M.: Surg., Gynec. & Obst. **80**:1, 1945. (b) Abramson, D.; Lerner, D.; Shumacker, H., and Hick, F.: Am. Heart J. **32**:52, 1946. (c) Leigh, O.: Ann. Surg. **124**:301, 1946. (d) Boland, F.; Claiborne, T., and Parker, F.: Surgery **17**:564, 1945. (e) Paddock, F.: New England J. Med. **34**:433, 1946. (f) Osborne, J., and Cowen, J.: Lancet 2:204, 1945. Friedman.[1a] Lesser.[1c]

The men complained of pain on walking and of hyperhidrosis. Often a patient alleged that he had to change his socks two or three times daily because of perspiration. The pain was located primarily in the balls of the feet, and as a result the men refused to put any weight on this part of their feet and walked with a characteristic shuffle. On arrival at the general hospital, they did not cover their feet with blankets or sheets, claiming that covering caused their feet to burn. This difficulty disappeared spontaneously within a few weeks. Many of the men described increased sensitivity to marked changes in temperature as compared with what they had experienced earlier in life.

What was most surprising was the contrast between the complaints of men who had lost tissue and those who had not. The latter were much more vociferous in complaining of their symptoms, especially of the hyperhidroses and pain.[8] On the other hand, it was not at all unusual to see a man with several gangrenous toes getting about with little complaint and even playing softball.

In contrast to the subjective symptoms, objective findings were lacking in the men who did not suffer loss of tissue. The pedal hyperhidrosis was accompanied almost invariably by manual hyperhydrosis. A study, in the same hospital, of the pulses and sweat responses failed to demonstrate any clearcut differences between a large group of patients with trench foot and other hospital patients not suffering from any peripheral vascular disease.[8a] Some cyanosis and edema were apparent on standing but were never marked enough to use as a differentiating point in diagnosis. The diagnosis of trench foot at this stage was based purely on the history.[8b]

Other investigators [9] have studied the clinical aspects of the disease and have, in general, encountered the same clinical symptoms. Stein and Dry [9a] have noted that no tests indicated any organic disease but that some tests suggested the possibility of functional derangement. Abramson and associates [7b] apparently depended primarily on the patients' subjective complaints. In a follow-up of their cases, Abramson and Shumacker [9b] concluded that sympathectomy was of no demonstrable value. Boland and co-workers [7d] were unable to distinguish between patients and controls with regard to skin reactions induced by changes of

8. (*a*) Silverman, J.: Ann. Int. Med. **22**:702, 1946. (*b*) Krause, L.: Personal communication to the author.

9. (*a*) Stein, I., and Dry, T.: Mod. Conc. Cardiovasc. Dis., 1946, vol. 15, no. 9. (*b*) Abramson, D., and Shumacker, H., in Proceedings of the Eighteenth Annual Meeting of the Central Society for Clinical Research, Chicago, Nov. 2-3, 1945. (*c*) Patterson and Anderson.[7a] (*d*) Abramson and others.[7b] (*e*) Leigh.[7c] (*f*) Boland and others.[7d] (*g*) Paddock.[7e] (*h*) Osborne and Cowen.[7f]

temperature in the more chronic stages of the disease. Paddock [36] noted certain minimal changes in temperature reactions, but these could have been due to a preexisting neurovascular imbalance which in itself would predispose toward susceptibility to trench foot.

In the men who had lost tissue, the areas immediately adjacent to the demarcation zone were fibrous, with splotchy cyanotic and erythematous areas. At amputation the surgeons often remarked about the lack of elasticity of the tissues. However, beginning about 3 to 4 cm. proximal to the demarcation zone, the tissues appeared grossly normal.

Characteristically, the symptom of pain in the balls of the feet was relieved when the men were induced to walk correctly. A typical example is case 183928. For about two years the patient had refused to place any weight on the ball of his right foot and had walked with a marked limp somewhat similar to that seen in a case of peroneal palsy. When he was reassured and began to walk correctly, a tremendous improvement was apparent in a few days. The edema of other patients who had consistently refused to get out of bed was also relieved when they were persuaded to walk.

MATERIAL AND METHODS

Of the 15 patients studied, 7 had lost tissue varying from a part of a toe to two or three phalanges of all the toes. The amputation specimens will be made the subject of another report. In the remaining 8, whose involvement was of the milder type, the gangrenous tissues had been exfoliated without any permanent loss of tissue.

All of the patients were combat soldiers. Fourteen had been exposed to cold wet weather in the winter of 1944-1945 in the European Theater of Operations. One (183928) had received his injury in the Attu campaign of 1943, and biopsy was done about two years after his evacuation.

Control biopsy specimens were obtained from volunteers, none of whom had been overseas. All had passed recent physical and laboratory examinations, preparatory to entering medical school. They were in the same age group as the patients, but there was not as great a spread between the youngest and the oldest as among the patients.

The biopsy tissues were obtained from two locations—the inner side of the foot medial to the phalangeal-metatarsal joint and the lower one fourth of the leg medial to the tibia. Each specimen included epithelium, dermis and a small amount of subcutaneous tissue but no muscle. Each patient, as well as each control, was kept in bed for at least ten hours prior to biopsy. Each had the same preanesthetic medication (morphine and atropine) and was operated on under intravenous pentothal anesthesia by the same surgeon.

The tissues were fixed for from four to six hours in Helly's fluid, embedded in pyroxylin (nitrocellulose) and cut serially at 8 to 10 microns. All tissues were stained with hematoxylin-eosin-azure II, Mallory-azan and orcein. In addition,

many of the slides were impregnated with silver by a modified Foot technic for reticular fibers.[10]   Specific nerve stains or impregnations were not done.

MICROSCOPIC DESCRIPTION

Tissues from Controls.—Because certain characteristics thought of as abnormal if encountered elsewhere in the body were found to be present in the controls, a histologic description of the tissues of the normal foot seems warranted in order to emphasize these unusual phenomena.   Another important point noted was the variability of a structure from person to person and even in the same person.   This included almost any of the structures—for example, the number of sweat glands or the amount of collagen in a nerve (figs. 8 and 9).

The epidermis was of the usual type, with the four classic layers fairly well marked.   The size and the number of melanin granules in the basal germinative layer varied, as did also the amount of intercellular and extracellular fluid.   Mitoses were seen only once or twice in slides of the eight biopsy specimens.   No sign of leukocytic invasion of the epidermis was seen in any control.

Degenerated cells were extremely rare.   There was some variation in the number, the size and the depth of the rete pegs from control to control and even in the same control.   In none were they absolutely uniform in size, shape and frequency.   The epidermis was always separated from the underlying papillary layer by a clearcut reticular fiber basement membrane.

Neither the size nor the number of sweat glands was uniform, and the amounts of secretory granules and especially the height and the staining quality of the secretory cells were subject to wide variations.   Basophilic granules filled the lumen and the distal ends of some cells and were entirely lacking in others.   The nuclei were of different heights and degrees of turgidity.   There was usually much less variation in the duct cells and in the numbers of myoepithelial cells and vessels in the fatty connective tissue supporting these glands.

The connective tissue elements, vessels, cells and fibers, were always subject to wide variation from control to control and often in the same slide.

The number, the size and the dilatation of the papillary capillary loops were extremely variable.   In some instances, papilla after papilla was filled with distended capillary loops.   The ascending and descending loops were always identical morphologically, and it was impossible to determine which was the ascending and which the descending loop except by use of serial sections.   The reticular fiber network of the subpapillary arteries and veins continued over on the papillary capillary loops.   The elastic fibers never followed the vessels superficially to the subpapillary plexus and in the papillae had no direct relation to the capillaries of the papillae.

The arteries in the subcutaneous layer differed in size and contractility but were all qualitatively similar in that the usual layers of intima, muscularis and adventitia were quite clear.   The walls of the vessels were heavy in some subjects, delicate in others.   There was always a slight variation in the amount of edema and intercellular fibers between the individual smooth muscle cells of the muscularis, although there were never enough fibers in the media to make it impossible to differentiate the arteries from the veins.

The arteries of the subcutaneous plexus sent branches superficially through the reticular layer to form the subpapillary plexus.   As these coursed through the

10. Buchsbaum, R., and Loosli, C.: Methods of Tissue Culture in Vitro and Outlines of Histological Methods, Chicago, University of Chicago Press, 1936.
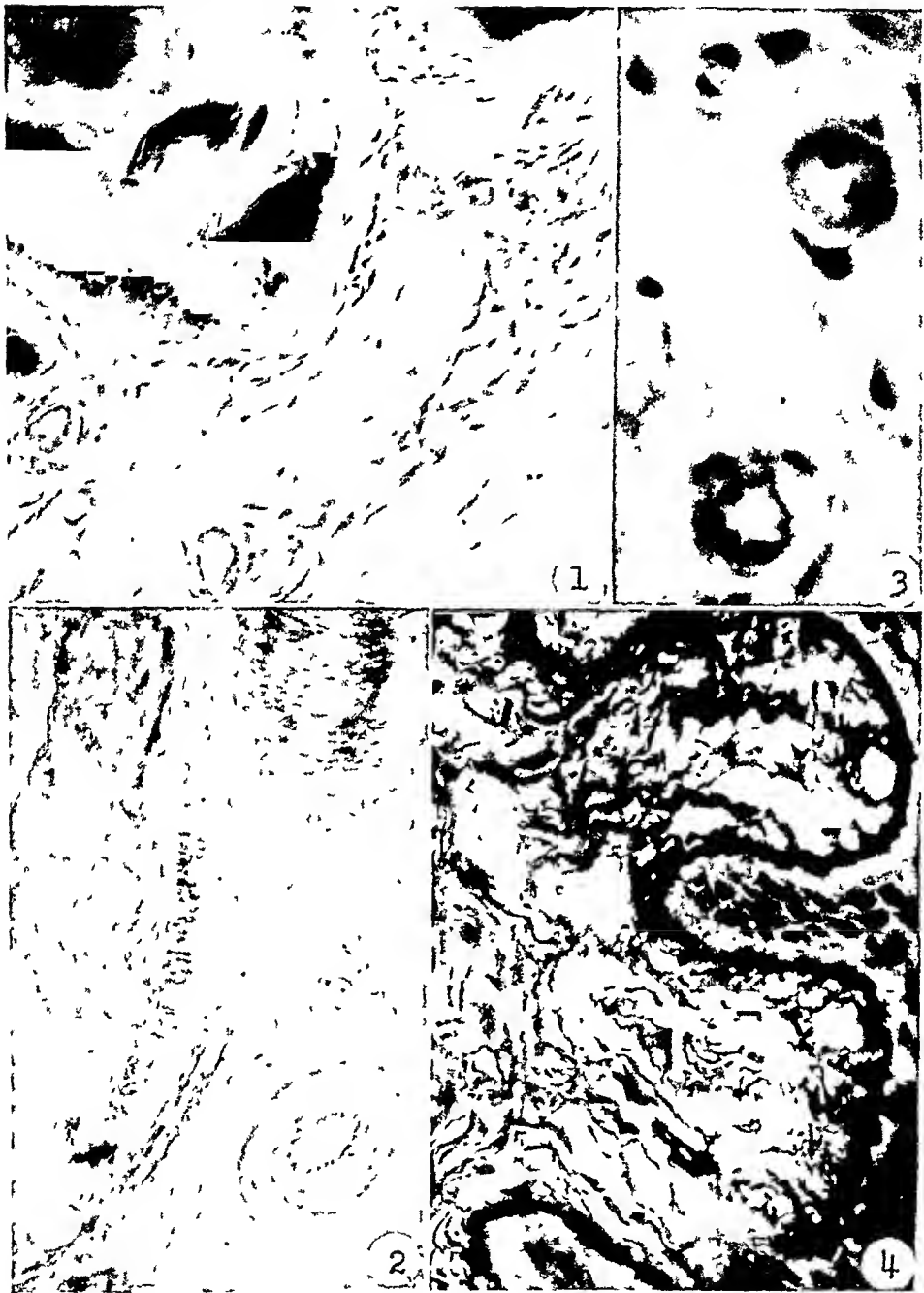
Fig. 1 (patient).—Moderate but variable amounts of epidermal intercellular edema. Marked dilatation of capillary loops in papillae and of subpapillary vessels. No inflammatory cells. Hematoxylin-eosin-azure II; × 210. Army Institute of Pathology negative 98565.

Fig. 2 (patient).—Heavy-walled subcutaneous vessels. Small amount of neural and perineural collagen. Hematoxylin-eosin-azure II; × 135. Army Institute of Pathology negative 98559.

Fig 3 (control).—Sclerotic small artery and, below and to left, normal small artery in subpapillary plexus. Hematoxylin-eosin-azure II; × 1,000. Army Institute of Pathology negative 98569.

Fig. 4 (patient).—Normal reticular fibers in papillae with perfectly preserved basement membrane. Silver; × 500. Army Institute of Pathology negative 98615.
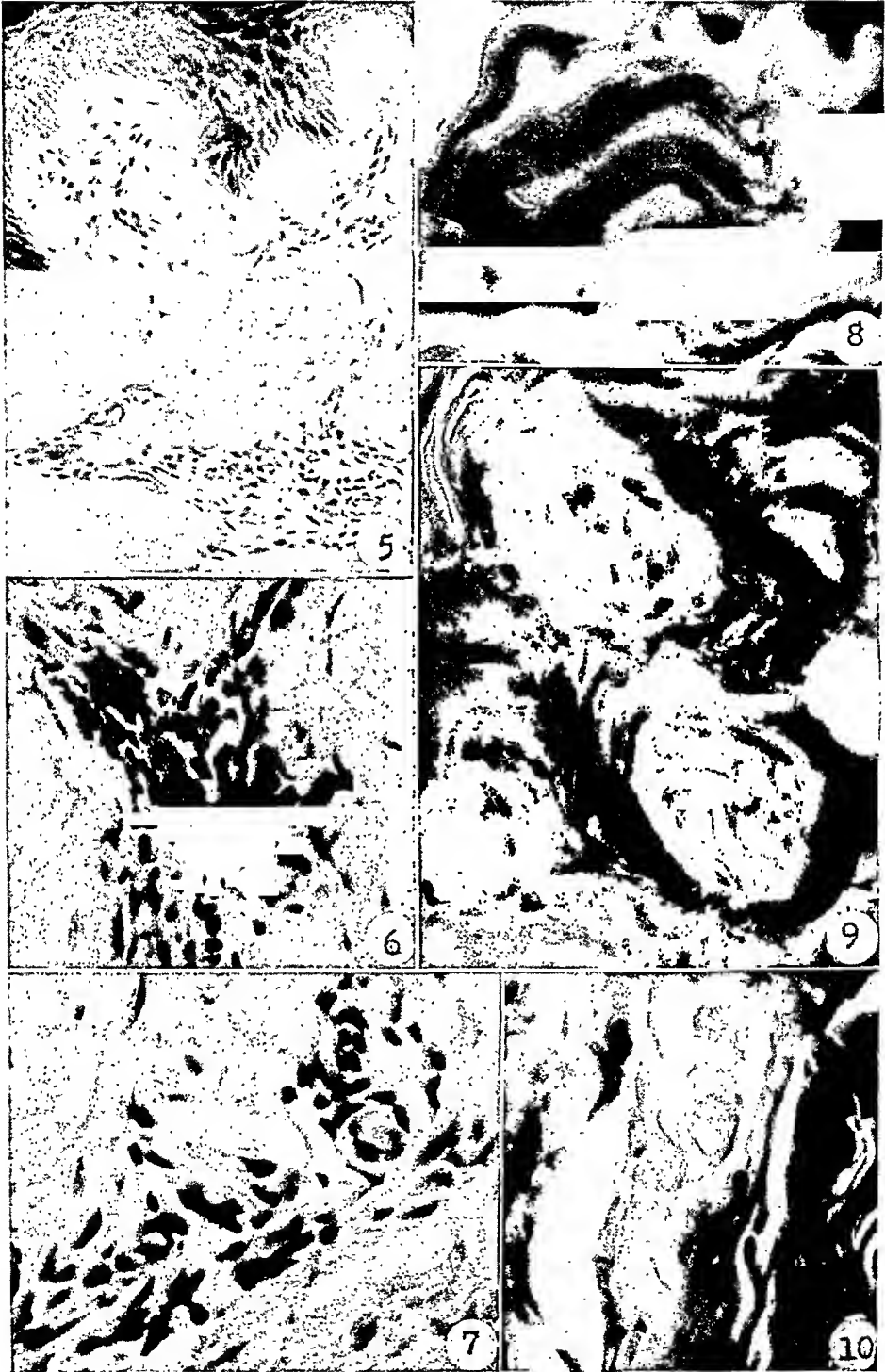
(See legends on opposite page)

reticular layer, they lost their definite adventitial coat, and the internal elastic membrane became irregular and discontinuous. In the subpapillary plexus the arteries were reduced to an endothelium and a single irregular layer of smooth muscle cells (fig. 3). Usually an internal elastic membrane was lacking, but occasionally it was still demonstrable. The reticular fiber sheath was always quite heavy, heavier than about the veins. There was a great deal of variation in the amount of sclerosis in these arteries (fig. 3).

The intima and the media of the subcutaneous veins were always mixed so that one might speak of them collectively as an intima-media. The elastic fibers were scattered through the intima-media and did not form an internal elastic membrane, but an external elastic membrane was usually quite prominent. The subcutaneous veins sent branches through the reticular layer to form several plexuses in the reticular layer and finally the subpapillary plexus. The veins of the subpapillary layer never had any smooth muscle and were recognized primarily by their reticular and elastic fibers.

The fibers forming the scaffolding of the connective tissue of the skin were heavy and numerous.

Reticular fibers were found only in certain locations. In the subcutaneous layer they formed a network about individual fat cells, sweat glands and vessels. In the reticular layer they were found only perivascularly. In the papillary layer they formed the basement membrane and capillary sheaths and were hardly to be seen at all in the rest of the papillae.

The collagenous fibers of the reticular layer were extremely heavy, dense and somewhat hyalinized (figs. 3 and 9). There were variations in staining, so that in the Mallory-azan stain a few of the fibers stained red; in most cases they consistently stained blue. There was always a contrast in the collagen of the reticular and papillary layers in that the fibers of the latter were always finer and looser and there was more interfibrillar fluid.

The elastic fibers were fairly evenly distributed in the reticular layer, but in the subcutaneous layer they were sparse. They usually adopted a corkscrew appearance in the papillary layer, between vessels and basement membrane, and also formed a network anastomosing just superficial to the basement membrane.

Numerous inflammatory cells were seen about the subpapillary plexus and about the branches of vessels coming from the subcutaneous plexus near the sub-

---

Fig. 5 (control).—Perivascular inflammation. Twisting capillary loops; moderately dilated vessels. Normal collagen. × 450. Army Institute of Pathology negative 98619.

Fig. 6 (control).—Marked perivascular inflammation. Hematoxylin-eosin-azure II; × 450. Army Institute of Pathology negative 98567.

Fig. 7 (control).—Moderate perivascular inflammation. Hematoxylin-eosin-azure II; × 545. Army Institute of Pathology negative 98610.

Fig. 8 (control).—Small amount of perineural fibrosis; rather marked endoneural fibrosis. Mallory-azan. Army Institute of Pathology negative 98612.

Fig. 9 (control).—Marked perineural fibrosis. Normal small artery, although slightly dilated. From same patient as figure 8. Mallory-azan; × 545. Army Institute of Pathology negative 98613.

Fig. 10 (patient).—Minimal amount of perineural fibrosis. Average amount of collagen in endoneurium. Mallory-azan; × 660. Army Institute of Pathology negative 98611.

papillary plexus. There was a wide variation among the patients and the controls in this respect, some not having any inflammatory cells, others having so many that the areas would certainly have been called abnormal in any other circumstances (figs. 5, 6 and 7). These cells were primarily lymphocytes and monocytes, although in a few instances they were macrophages and fibroblasts. In some controls there were numerous mast cells in the same locality; in others, none at all. The granules of the mast cells were rather small and ill defined. There was no evidence of mononuclear invasion of any structure.

Another striking inconsistency was in the amount of collagenous and reticular fibers seen in the nerves (figs. 8 and 9). In one person the perineurium had so much collagen that it was almost one-third as thick as the nerve; elsewhere in the same person the perineurium was so fine as to be hardly recognizable (fig. 8). The endoneural fibers likewise varied from the finest to the coarsest. There was no correlation between the amount of fibers and any other characteristic on the slide.

There was nothing unusual in the fatty tissue of the subcutaneous layer.

Tissues from Patients.—A comparison of the biopsy specimens from patients and controls failed to reveal any significant difference between the two groups in regard to any structure examined (figs. 2, 4 and 10). However, special nerve fiber preparations were not made, and the biopsy specimens were not deep enough to include muscle. But there were pathologic features in tissues from a certain few of the patients which were not duplicated in any of the controls and which were probably related to the past history of trench foot.

In case 183435 an unusually large amount of edema was present in the papillary layer of the dermis. It is probable that the patient's vessels were unusually thick and prominent (fig. 2). There was an unusually large number of prominent vessels in the subpapillary plexus and in the connective tissue of the papillary layer of the dermis in case 183430 (fig. 1). It must be emphasized that these variations were minute, and it was only after the specimens had been compared with the controls repeatedly that the distinctions were established. The clinical courses were similar in that both patients refused to get out of bed and walk as did the other patients. The biopsy specimens were taken when both were still in bed with doughy, edematous feet, painful to palpation. Trench foot was moderate in both patients; there was no loss of tissue, and improvement was rapid after the patients began to walk.

In case 183434, in which trench foot was mild, the intima of a vein in the superficial part of the reticular layer of the dermis was greatly thickened and was quite as abnormal as any of the vessels seen in the amputated specimens. However, there was nothing unusual in any other part of this biopsy specimen.

Surprisingly enough, no pathologic changes were found in any of the specimens from the men who had lost tissue. In 1 case the second or third phalanx of each toe was amputated at the same time that the biopsy specimens were taken. The biopsy specimen taken only a few centimeters from the amputation line failed to show any pathologic features. Apparently, the marked pathologic changes occurring in the zone of regeneration of the amputated specimen did not extend proximally far enough to involve the site of removal of biopy tissues in any of the patients.

In both patients and controls occasionally there were certain morphologic features which were characteristic enough to serve as a means of identifying the source of the tissues. For example, one man had extremely heavy-walled arteries and veins. These structures were all large and gross as compared with those of any of the other men (fig. 2). Some of the men had more pigment in the stratum germinativum of the epidermis than others. There were variations in the number

and the prominence of vessels from one man to the next. In brief, no two specimens were exactly alike, although, as mentioned previously, the differences were too inconsistent to serve as a means of separating the controls from the patients.

COMMENT

In previous reports of biopsies of the chronic stages of trench foot and immersion foot, certain pathologic features have been described. Boland and associates [7d] described vascular dilatation, perivascular inflammation and increase of collagen occurring about ninety days after the onset of symptoms as compared with control tissue taken post mortem from patients with previously normal feet. White and Warren [2c] noted increased thickening of the corium, scarcity of skin appendages, hyalinization of collagen, smoothing of rete pegs, perineural fibrosis and fibrosis extending along individual muscle fibers and groups of fibers. Paddock [7e] noted minor swellings of axons and minimal neural fibrosis. Ungley and Blackwood [11] described changes in the larger nerves and vessels. However, these investigators [12] did not have the opportunity to obtain control biopsy specimens from the same location in healthy living normal persons and so were not able to take into account the specialized structure of tissues of the feet. Unfortunately, none of my biopsy specimens was taken deep enough to contain muscle, and so no opportunity was available to study the deeper structures.

Blackwood and Russell,[13] after observing experimental lesions of rats simulating trench foot, emphasized the deep neural and muscular changes and failed to find anything significant in the chronic stages as far as the blood vessels were concerned. Denny-Brown and co-workers [14] stated that the neural lesions produced experimentally under conditions similar to those causing trench foot tend to undergo complete repair within ninety days.

In regard to pernio, McGovern, Wright and Kruger,[4] from their observations of biopsy material, described changes in the vessels· and connective tissues which were similar to, if not identical with, those seen in the amputated specimens from patients with trench foot. However, the appearance of the tissue in their figure 16 seems within normal range.

The minimal, perhaps debatable, pathologic changes encountered in specimens from 2 patients with a mild form of trench foot should not be

11. Ungley, C. C., and Blackwood, W.: Lancet 2:447, 1942.

12. White and Warren.[2c] Boland and others.[7d] Paddock.[7e] Ungley and Blackwood.[11]

13. Blackwood, W., and Russell, H.: Edinburgh M. J. **50**:385, 1943; **52**:160, 1945.

14. Denny-Brown, D.; Adams, R. D.; Brenner, C., and Doherty, M.: J. Neuropath. & Exper. Neurol. 4:305, 1945.

interpreted in the same light as the changes in tissues from the other patients. By refusing to walk, these 2 patients retarded the normal healing processes; thus the same stage in repair had not been attained as in the tissues of the men who were already actively ambulant at the time of biopsy.

One objection may be raised to the method by which the biopsy tissues were taken. Since both patients and controls were kept in bed for the ten hours prior to operation, any latent deficiency of the functioning of the neurovascular system would have been masked in the same way as resting in bed will prevent distention of varices in varicose veins of the lower limb. Perhaps if the controls and the patients had been made to walk up to the moment the biopsy specimens were taken, the tissues might have been more edematous and the vessels more dilated. But the strain occasioned by walking would hardly have been expected to change the basement membrane or the nerves.

The interpretation of the functional state of the patients in terms of the biopsy results was extremely difficult. Certainly, neither the biopsies nor the objective clinical findings revealed anything at all which could explain the clinical symptoms. The speed with which these symptoms were alleviated indicated that little of pathologic significance would be found after the men had been ambulant for some time. However, it is realized that absence of microscopic lesions does not exclude the possibility of functional derangement. Furthermore, the excision of specimens was not deep enough to exclude deep nerve and muscle disease, although the symptoms and the clinical course were such as not to point to changes of muscle or deep tissues.

The individual differences found from patient to patient and even in the controls were interesting. It seemed as though no two skins were absolutely identical. In the same way the individual reactions to cold wet weather were variable. In only a small percentage of any unit did trench foot develop. Some men were obviously more susceptible than others to the environmental changes.[7a, f] In a report of carefully controlled animal experiments, Böttcher [15] has referred to the marked individual variation in susceptibility of rats to cold. Since there were morphologic variations in the controls, it seems quite possible that there may also be physiologic variations in the neurovascular system, and perhaps both of these will serve to indicate in the future who will and who will not be an early candidate for trench foot.[7f]

SUMMARY

Biopsies were performed on 15 men who had absolutely clearcut histories of uncomplicated trench foot. Of these 15 men, 7 had lost some

---

15. Böttcher. H.: Virchows Arch. f. path. Anat. **312**:464, 1944.

tissue. The biopsy specimens from these patients as a group failed to reveal any pathologic changes when compared with those from 8 controls of the same age group.

Certain morphologic features—mild hyalinization of nerves, perineural fibrosis, perivascular inflammation and hyalinization of collagen—which would ordinarily be considered abnormal were found in both patients and controls.

Many of the individual subjects exhibited slight variations from the average which indicated the possibility that variation may occur in physiology and consequently in susceptibility to environmental changes.

In treatment the best results were obtained by inducing the patients to walk as early as possible.

PATHOLOGIC SIGNIFICANCE OF THE DUCTUS ARTERIOSUS

Its Relation to the Process of Arteriosclerosis

LESTER S. BLUMENTHAL, M.D.[*]

ROCHESTER, MINN.

A SURVEY of mortality rates of recent years[1] reveals that diseases of the heart and the blood vessels now head the list of causes of death. The primary unsolved problem in pathology continues to be that of arteriosclerosis.[2] This condition serves as a basis for most of the clinical diseases of the heart and the blood vessels. With this in mind, I have studied a small aspect of the gross problem.

One of the vascular structures of the body where a phenomenon resembling arteriosclerosis of later life develops early is the fetal ductus arteriosus, closure and subsequent obliteration of which occur soon after birth. Robertson[3] expressed the opinion that the closure of the ductus arteriosus is a form of arteriosclerosis and that in the processes of its closure some processes that resemble arteriosclerosis of later life might be observed. Winternitz and his associates[4] studied the "adaptive" changes that occur normally in three groups of blood vessels. These changes are the obliteration of the fetal vessels at birth, the changes in the uterine artery during and after pregnancy, and the obliteration of large pulmonary vessels that traverse tuberculous cavities. He demonstrated increased vascularity of the walls of the vessels in these "adaptive" changes, together with many other changes, including hemorrhage and calcification, which he found in arteriosclerotic vessels. He thought this demonstration increased the significance of the vascularity of arteriosclerotic sites and emphasized anew the validity of the idea that pathologic changes have their less violent counterparts in normal physiologic processes.

The implied relationship between the closure of the ductus arteriosus and the process of arteriosclerosis led to the present investigation, to

* Fellow in Medicine, Mayo Foundation.

1. Twenty-Five Years of Health Progress, New York, Metropolitan Life Insurance Company, 1937.

2. Arteriosclerosis, editorial, J. A. M. A. **128**:517, 1945.

3. Robertson, H. E.: Personal communication to the author.

4. Winternitz, M. C.; Thomas, R. M., and LeCompte, P. M.: The Biology of Arteriosclerosis, Springfield, Ill., Charles C Thomas, Publisher, 1938.

see whether there is a parallel between arteriosclerosis and the closure of this vessel and to determine whether any further information can be obtained about the etiologic nature of arteriosclerosis.

THE DUCTUS ARTERIOSUS

The ductus arteriosus has received attention recently because heroic surgical procedures have been devised to correct persistent patency of the vessel.[5] The literature contains numerous excellent reports dealing with the manner of the normal closure at birth. These include embryologic,[6] anatomic,[7] histologic[8] and physiologic studies.[9] Most of the investigations were undertaken to substantiate one or another theory of the closure of the ductus; however, none attempted to correlate this process with the arteriosclerosis of later life.

5. Gross, R. E.: J. A. M. A. **115**:1257, 1940. Gross, R. E., and Hubbard. J. P.: ibid. **112**:729, 1939. Harrington, S. W.: Proc. Staff Meet., Mayo Clin. **18**:217, 1943. Keys, A., and Shapiro, M. J.: Am. Heart J. **25**:158, 1943. Touroff, A. S. W.: ibid. **25**:187, 1943.

6. (a) Arey, L. B.: The Vascular System, in Developmental Anatomy: A Text-Book and Laboratory Manual of Embryology, ed. 3, Philadelphia, W. B. Saunders Company, 1934, chap. 11, pp. 280-331. (b) Congdon, E. D.: Contrib. Embryol. **14**:47, 1922. (c) Gray, H.: Anatomy of the Human Body, ed. 23, edited by W. H. Lewis, Philadelphia, Lea & Febiger, 1936. (d) Heuser, C. H.: Contrib. Embryol. **15**:121, 1923.

7. (a) Cunningham, D. J.: Text-Book of Anatomy, ed. 8, New York, Oxford University Press, 1943. (b) Eaton, A. W.: Unpublished data. (c) Gräper, L.: Ztschr. f. Anat. u. Entwcklngsgesch. **61**:312, 1921. (d) Jager, B. V., and Wollenman, O. J.: Am. J. Path. **18**:595, 1942. (e) Morris, H.: Morris' Human Anatomy: A Complete Systematic Treatise, ed. 10, edited by J. P. Schaeffer, Philadelphia, The Blakiston Company, 1942. (f) Noback, G. J., and Rehman, I.: Anat. Rec. **81**:505, 1941. (g) Roeder, H.: Arch. f. Kinderh. **33**:147, 1902. (h) Strassmann, P.: Arch. f. Gynäk. **45**:393, 1894. (i) Gray.[6c]

8. (a) Barnard, W. G.: St. Thomas's Hosp. Rep. **4**:72, 1939. (b) Boyd, J. D.: J. Anat. **75**:457, 1941. (c) Costa, A.: Cuore e circolaz. **14**:546, 1930. (d) da Cunha, M. L.: Fac de med. de São Paulo **2**:245, 1927. (e) Kennedy, J. A., and Clark, S. L.: Anat. Rec. **79**:349, 1941. (f) Mělka, J.: Anat. Anz. **61**:348, 1926. (g) Swensson, A.: Ztschr. f. mikr. anat. Forsch. **46**:275, 1939. (h) Takino, M., and Watanabe, S.: Arch. f. Kreislaufforsch. **2**:18, 1937. (i) von Hayek, H.: Ztschr. f. Anat. u. Entwcklngsgesch. **105**:15, 1935. (j) Footnotes 7 b, c and d.

9. (a) Barclay, A. E.; Barcroft, J.; Barron, D. H., and Franklin, K. J.: Brit. J. Radiol. **11**:570, 1938; (b) **12**:505,1939. (c) Bettinger, H.: Centralbl. f. allg. Path. u. path. Anat. **54**:289, 1932. (d) Kennedy, J. A.: Am. J. M. Sc. **204**:570, 1942. (e) Kennedy, J. A., and Clark, S. L.: Am. J. Physiol. **136**:140, 1942. (f) Kowalski, W.: Virchows Arch. f. path. Anat. **233**:191, 1921. (g) Linzemeier, G.: Ztschr. f. Geburtsh. u. Gynäk. **76**:217, 1915. (h) Rauchfuss, C.: Virchows Arch. f. path. Anat. **17**:376, 1859. (i) Rehman, I.: Abstracted, Anat. Rec. (suppl., no. 2) **76**:47, 1940. (j) Schanz, F.: Arch. f. d. ges. Physiol. **44**:239, 1889. (k) Wells, H. G.: Am. J. M. Sc. **136**:381, 1908. (l) Footnote 7 b, c and d. (m) Footnote 8 a, e, f, g and i.

Kennedy and Clark [9e] presented a detailed description of the vasa vasorum of the ductus arteriosus and found these to be present in the outer portion of the media at birth. That portion of the wall of the ductus that lacked these vessels was the same portion in which the most marked degenerative changes occurred. They therefore postulated that after physiologic closure of the lumen of the ductus the central part is no longer able to obtain a supply of blood and that, because of this, degeneration occurs.

MATERIAL AND METHODS

The material of the present study was obtained from a series of 175 postmortem specimens. The specimens were obtained from fetuses and from persons of various ages. The oldest was a man aged 96 years. The ductus arteriosus or the ligamentum arteriosum arteriae pulmonalis was removed intact, fixed in solution of formaldehyde U. S. P., sectioned, and stained with hematoxylin and eosin. Many sections were also stained with the Van Gieson or with the elastin H stains to demonstrate more clearly the elastic, muscular and connective tissues.

RESULTS

The only practical way to present the findings is to describe specimens representative of each age group. The specimens representing fetuses in the earlier stages of development had the basic structure found in those representing fetuses at full term. There was little change in this until after birth. From birth to the first few weeks of life the most extreme alterations were observed. By that time degeneration had begun. The only change that occurred later was an increase of the degeneration of the tissues, but even with definite degeneration the basic structure of the ductus could be distinguished with ease.

Fetuses.—The youngest fetus was just past the fifth month. The cross section of the ductus arteriosus showed a layer of normal endothelium with a single-layered internal elastic lamina below. Immediately adjacent to this there was wide-meshed areolar-like tissue. This was occupied in certain locations by wandering cells and by dark-staining small spindle-shaped cells lying in a direction perpendicular to the internal elastic layer. Deeper in this areolar-like tissue the smooth muscle cells of the media at first appeared separated by tissue spaces and tiny fibrils, but as the adventitia was approached, their arrangement became more compact and assumed a definitely circular aspect. The outer layers of the media blended imperceptibly with the adventitia, without an intervening external elastic lamina. The media was strikingly free from the thick bands of elastic tissue that are seen in the pulmonary artery and the aorta.

In some specimens there were elevated plaques or mounds such as are seen grossly when the intima of a patent ductus arteriosus is examined at birth. These mounds are often made up of cells which closely

resemble the smooth muscle cells of the media, and there seems to be a "streaming in" of these cells from the media to the mounds. Where the lumen was reduced in diameter, the mounds were higher and more compact. The media also was more compact, and the tissue spaces between the muscle cells were much narrower. This probably was evidence of active muscle contraction.

Infants from Birth to Twelve Days of Age.—The wall of the duct was the same in this group as in the premature group. The mounds were more numerous and larger. The internal elastic layer was split and reduplicated into two or three thin strands. The media was composed of thicker bundles of circular and oblique muscle cells, with wide tissue spaces between these bundles. There was no evidence of degeneration in any specimen. The tissue of the mounds and the smooth muscle of the media stained yellow with the Van Gieson stain and contrasted markedly with the red-staining fibrous connective tissue of the adventitia.

Infants from Twelve Days to Two Months of Age.—This group, which included specimens from 8 infants, was the most interesting to observe. A gradual progression of degenerative changes took place in the ductus arteriosus, beginning on the twelfth day. On the twelfth day the lumen of the ductus was contracted to a narrow slit. There was a ghostlike washed-out appearance, as if infarction had occurred in the intima and the inner part of the media.

The specimen from the infant 16 days old (*A* in figure) showed the tissue to be compressed, with almost complete obliteration of the tissue spaces of the mounds. There were a decrease in the number of deep-staining nuclei, an increase in the amount of elastic fibrils and marked ischemia of the entire mound. When Van Gieson stain was applied, the tissue took on a pink or a red tint, which is evidence of fibrosis. There was no actual necrosis or deposition of calcium.

The next step in the degenerative process was seen in the specimen (*B* in figure) from an infant 25 days old. Generalized washed-out appearance and lack of nuclei were observed in the cells of the intima and the inner part of the media. The muscle cells were paler, and much fibrous connective tissue was interspersed among them. One area showed early necrosis of the intima.

As the age increased, the area of ischemia increased, and more fibrous connective tissue replaced normal muscle tissue. This process of degeneration gave the impression that as the ductus contracts the imbibition of blood and plasma is decreased with resultant ischemia of the tissues. If the degeneration progresses slowly, the specialized smooth muscle is gradually replaced by fibrous connective tissue. It may be that this ischemia acts as a stimulus for the elastic tissue to disrupt or split up rather than to degenerate, probably because elastic tissue is a
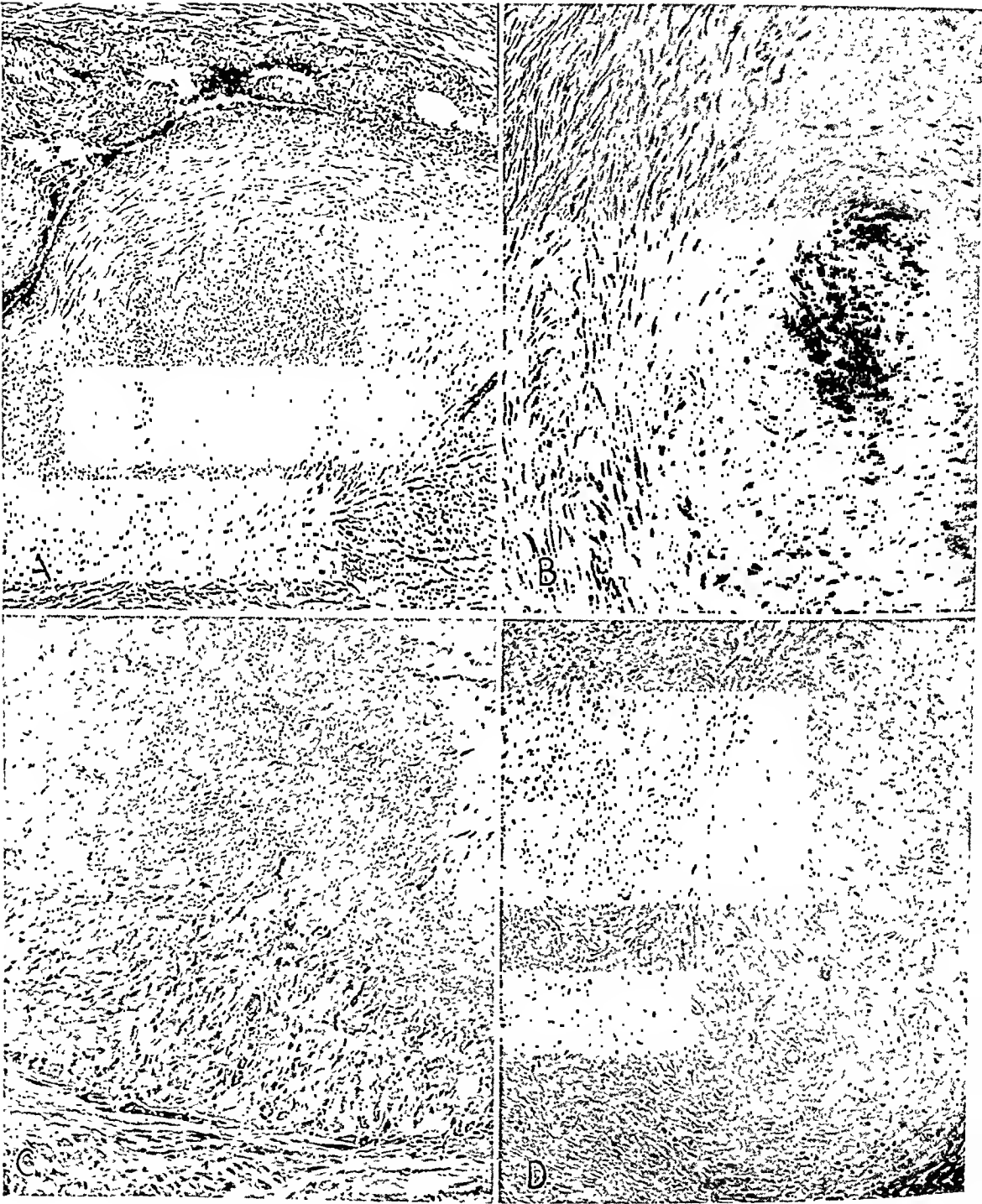
Cross sections of ligamentum arteriosum stained with hematoxylin and eosin: *A*, from an infant 16 days of age (× 65); *B*, from an infant 25 days of age (× 150); *C*, from a woman 22 years of age (× 110); *D*, from a man 56 years of age (× 80).

less specialized tissue than muscle tissue and therefore needs less oxygen to exist. If the ischemia is excessive, there may be actual necrosis of tissue instead of replacement by tissue of a lower order.

Infants from Two Months to One Year of Age.—The degenerative changes continued to increase. Most specimens contained areas of necrosis and calcium deposits. The tissue adjacent to the slitlike remnants of the lumen appeared to be new and alive, and many red blood cells in good state of preservation remained in the lumen. Small capillaries were often seen adjacent to the lumen. These may represent efforts to recanalize the tissue. The outer layers of muscle retained their normal appearance, but isolated nuclei and an occasional clump or bundle of muscle cells were all that remained of the inner layers.

After the Age of One Year.—Van Gieson stain showed a gradual increase of the intensity of the fibrosis until the color of the new tissue almost matched the deep red–staining fibers of the adventitia. The only significant change was the decrease in smooth muscle at the outer part of the media. This gradually became reduced to single strands in a circular manner and definite clusters of cells in certain areas. After many sections presenting the same cluster-like arrangement of these muscle cells were observed it became evident that there was a definite reason for this arrangement. It was found that these clusters were adjacent to small blood vessels and that the clusters were usually absent in areas devoid of capillaries. *C in the figure demonstrates this arrangement well.* These findings give support to the idea that the process of fibrous replacement of the ductus arteriosus is of an ischemic or anoxemic nature. The internal elastic lamina remained intact and prominent even in the older specimens. Elastic fibrils gradually increased in number. Before the tenth year the fibrils invaded the intima, but later they also extended throughout the media.

Another interesting finding in several specimens from older persons was formation of cartilage. *D in the figure shows this cartilage well.* The specimen was obtained from a man aged 56 years. The oldest persons from whom specimens were obtained were 90 and 96 years of age. Extreme degeneration and much deposition of calcium were observed in these two specimens, but under low magnification the basic structure was plainly evident.

<div align="center">COMMENT</div>

The primary purpose of this study was to try to add to the understanding of the genesis of arteriosclerosis.

The closure and subsequent obliteration of the ductus arteriosus is a phenomenon occurring naturally in most newborn mammals. This process is a form of arteriosclerosis that occurs with a physiologic purpose. It was therefore believed that a study of the histologic changes

involved in this metamorphosis might disclose certain processes parallel to those that occur in arteriosclerosis of later life.

Most of the authors who have studied arteriosclerosis from the standpoints of pathology and etiology carefully agree in their descriptions of the histologic changes involved but do not agree in their conceptions of the etiologic factors. They are unwilling to concede that arteriosclerosis is an unavoidable accompaniment of the aging process, for to do so would be fatalistic and would make the ultimate prognosis hopeless. Most authors agree that processes which accompany the wear and tear of life play a large part. Others believe that infections and their sequelae produce the underlying change. Still others stress blood lipids as the primary causative agent.

Hueper [10] presented an excellent review of the entire subject and proposed that anoxemia is a basic means through which any or all of the various agents may work to produce arteriosclerosis. According to this concept, localized low blood pressure leads to stagnation of the blood flow and thus to anoxemia, whereas elevation of the blood pressure is accompanied by vasoconstriction which reduces the blood flow to the vasa vasorum. He did not discuss the closure of the ductus arteriosus, but it has been shown in the present study that such a mechanism could also be active here. Kennedy and Clark [9e] touched on this concept after studying the vasa vasorum of the walls of the ligamentum arteriosum.

New surgical technics for the repair of persistent patency of the ductus arteriosus have stimulated much experimental investigation of the ductus. This has added new meaning to the older histologic descriptions of the prenatal and the postnatal state of the ductus. As a result of recent physiologic and roentgenographic studies, the closure of the ductus at birth has taken on a dynamic character, for now the vessels can be seen to contract and reexpand in response to various stimuli. This proof of actual muscular contraction allows observers to divide the closure of the vessel into two separate parts. The first is the active contraction of the muscle of the media at birth. The second is the maintenance of this state of contraction after birth when a definite sequence of histologic changes occurs. The changes probably begin soon after birth. Once they have occurred, the lumen will remain contracted even if the muscle of the media has a tendency to relax.

Histologic examination of the ductus arteriosus before birth reveals that it is a true muscular organ. The intimal mounds are probably composed of smooth muscle of a modified type. Examination of the vessel after birth shows that active muscle contraction has occurred.

Twelve days after birth an interesting series of degenerative changes occurs. It is instructive to follow this metamorphosis from the actively

---

10. Hueper, W. C.: Arch. Path. **38**:162, 245 and 350, 1944.

functioning ductus to the obliterated degenerated ligamentum arteriosum. It seems as if actual infarction has occurred in the intima and the media. It may be that the fetal ductus receives its blood supply from its lumen and its vasa vasorum and that after active muscle contraction of the wall of the ductus has been maintained for an appreciable time, this blood flow decreases to such an extent that actual anoxemia occurs. Thereafter, smooth muscle is gradually replaced by fibrous connective tissue and elastic tissue. Another finding that points to anoxemia is the necrosis and calcification occurring in certain specimens, beginning with the specimen from an infant aged 25 days. Most likely, the usual sequence is a gradual replacement of the muscle tissue by tissue of a lower order, but if more severe anoxemia occurs necrosis results.

These findings seem to lend support to Hueper's contention that anoxemia is the fundamental factor in the genesis of arteriosclerosis. The theories of wear and tear, senescence, toxicity, infection or hyper-lipemia as causative agents in arteriosclerosis are considered only briefly in this report.

SUMMARY AND CONCLUSIONS

The ductus arteriosus is a true muscular organ which on proper stimulation is capable of contracting strongly enough to obliterate its lumen. The stimulation is apparently the increase of the oxygen saturation of the arterial blood brought about by the onset of respiration in the newborn infant.[9d] After the contraction has been maintained for several days, actual histologic changes take place. Muscle tissue is replaced by tissue of a lower order of specialization. In some cases, the replacement is slow, and no necrosis occurs; however, in others rapid degeneration with necrosis and calcification takes place. The cause of this tissue replacement and degeneration appears to be relative anoxemia of the wall of the ductus brought about by contraction of the muscle. This contraction narrows the lumen, compresses the vasa vasorum and thus decreases the blood supply of the vessel wall.

These tissue changes occurring in the ductus arteriosus as the result of anoxemia seem to be similar to those observed by Hueper in arteriosclerosis. The work recorded in this paper, therefore, leads to the opinion that anoxemia plays a large part in arteriosclerotic processes and may possibly be the fundamental mechanism through which the various causal agents bring about arteriosclerotic changes.

Case Reports

PRIMARY MESOTHELIOMA OF THE PERICARDIUM

WILLIAM J. REALS, M.D., B. CARL RUSSUM, M.D.

AND

EDMUND M. WALSH, M.D.

OMAHA

THE RARITY of primary neoplasms of the heart and of its coverings, as well as the confusing clinical aspects of a patient with a tumor diagnosed as primary mesothelioma of the pericardium, led us to review the literature and to present a detailed clinical and postmortem report of a single case.

In the literature the terminology of this type of tumor is confused. Most European authors have referred to tumors of this type as "celothelioma," while Ewing[1] classified them as endothelioma, Yater[2] as endothelial carcinoma, and Geschickter[3] as mesothelioma. Klemperer and Rabin,[4] in reviewing tumors of mesodermal origin, stated that Krumbein (1924) found 30 different names for such tumors in medical literature.

The splanchnocele of the embryo gives rise to the serous cavities (peritoneal, pleural and pericardial) which are lined by persisting celomic epithelium, the mesothelium. In derivatives of the splanchnocele, celomic epithelium tends to persist, and the underlying mesoderm tends to form vascular connective tissue. Tumors arising from the pleura, the peritoneum and the pericardium, to which the name "mesothelioma" is given, show similar tendencies, with epithelial and fibrous constituents. In this paper, therefore, such tumors will be called mesothelioma.

Only 22 cases of mesothelioma of the pericardium have been recorded in medical literature. McDonald,[5] in presenting a case, credits Marchiafava (1875-1876) with recognizing and reporting the first example and cites cases of Dietrich, Okasaki, Ceelen, Sebastianoff and Winogradoff and Ervingham. Geschickter[3] reported 1 case and cited 11 collected by Bodon[6] (Chajutin, Redtenbacher, Godel, Schoppler, Loos, Hill, Tobiesen, Williams, Lazarus, Drysdale and Kaak). Single

From the Departments of Pathology and Medicine, Creighton University School of Medicine, and Creighton Memorial St. Joseph Hospital.

1. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940.

2. Yater, W. M.: Arch. Int. Med. 48:627, 1931.

3. Geschickter, C. F.: Am. J. Cancer 26:378, 1936.

4. Klemperer, P., and Rabin, C. B.: Arch. Path. 11:386, 1931.

5. McDonald, S.: J. Path. & Bact. 43:137, 1936.

6. Bodon, G.: Frankfurt. Ztschr. f. Path. 40:431, 1930.

cases were added by Dick,[7] who noted a case presented by ten Seldam,[8] and by Hines and Nolan.[9]

Microscopically, mesothelioma appears neither frankly endothelial nor frankly epithelial. It is cellular, with only a fine reticular supporting stroma, and the cells are arranged in bundles with a slight tendency toward whorl formation. Individual cells are oval to polyhedral, with large nuclei and prominent nucleoli, and mitoses are numerous.

In Dick's [7] case there was an irregular alveolar arrangement with tumor cells lining acinous spaces, but other authors (McDonald [5] and Ewing [1]) have not mentioned this distribution, nor was it seen in our case. Vascularity, however, may be a prominent microscopic feature.

REPORT OF A CASE

A. V., a 58 year old white farmer, was admitted, May 27, 1946, to Creighton Memorial St. Joseph's Hospital on the service of one of us (E. M. W.), complaining that he had suffered from severe cough, fulness of the chest and pain of the chest on exertion for six weeks. The illness seemed to follow a "heavy chest cold" with severe, poorly localized pain in the chest. On several occasions during the two weeks before admission he had coughed up small amounts of bright red blood. A physician in his home community had made a diagnosis of pneumonia and had treated him with intramuscular injections of penicillin, considerably relieving both the pain and the cough. Dyspnea and hemoptyses had, however, become worse, and the patient was therefore hospitalized for observation.

The general systemic history revealed only that the appetite had been poor since the illness began but that there had been no loss of weight. The past history was noncontributory.

The patient was well nourished and well developed. There was no cyanosis, although forced breathing was marked. The pupils reacted normally. The throat was injected, but there were no enlarged glands or other masses in the neck. There was fulness to percussion over the lower lobe of the right lung, with decreased vocal fremitus and breath sounds. A few scattered moist rales were heard posteriorly over the lower lobe of the left lung. The cardiac apex was located at the fifth interspace in the midclavicular line; the apical and radial heart rates were 86. No murmurs or irregularities were noted. The liver, the spleen and the kidneys were not enlarged, and there were no palpable masses in the abdomen. All reflexes were within normal limits.

On admission the urine was normal; the hemoglobin, 52 per cent; the red blood cell count, 2,620,000; the white blood cell count, 11,800; the differential count, monocytes 1 per cent, lymphocytes 19 per cent and neutrophils 80 per cent. Sputum showed no acid-fast organisms.

Roentgenograms of the chest suggested that there was thickening of the pleura in the right upper lung field with pleural effusion in the right lower lung field. After withdrawal of 500 cc. of thoracic fluid two days after admission, an area of increased density in the upper lobe of the right lung suggestive of atelectasis could be seen on additional films; a similar area was noted in the right cardiophrenic angle. The right side of the diaphragm was elevated (fig. 1). The thoracic

7. Dick, J. C.: J. Path. & Bact. **47**:43, 1938.

8. ten Seldam, R. E. J.: Geneesk, tijdschr. v. Nederl-Indië **76**:2703, 1936.

9. Hines, R. E., and Nolan, D. E.: M. Bull. Vet. Admin. **10**:82, 1942.
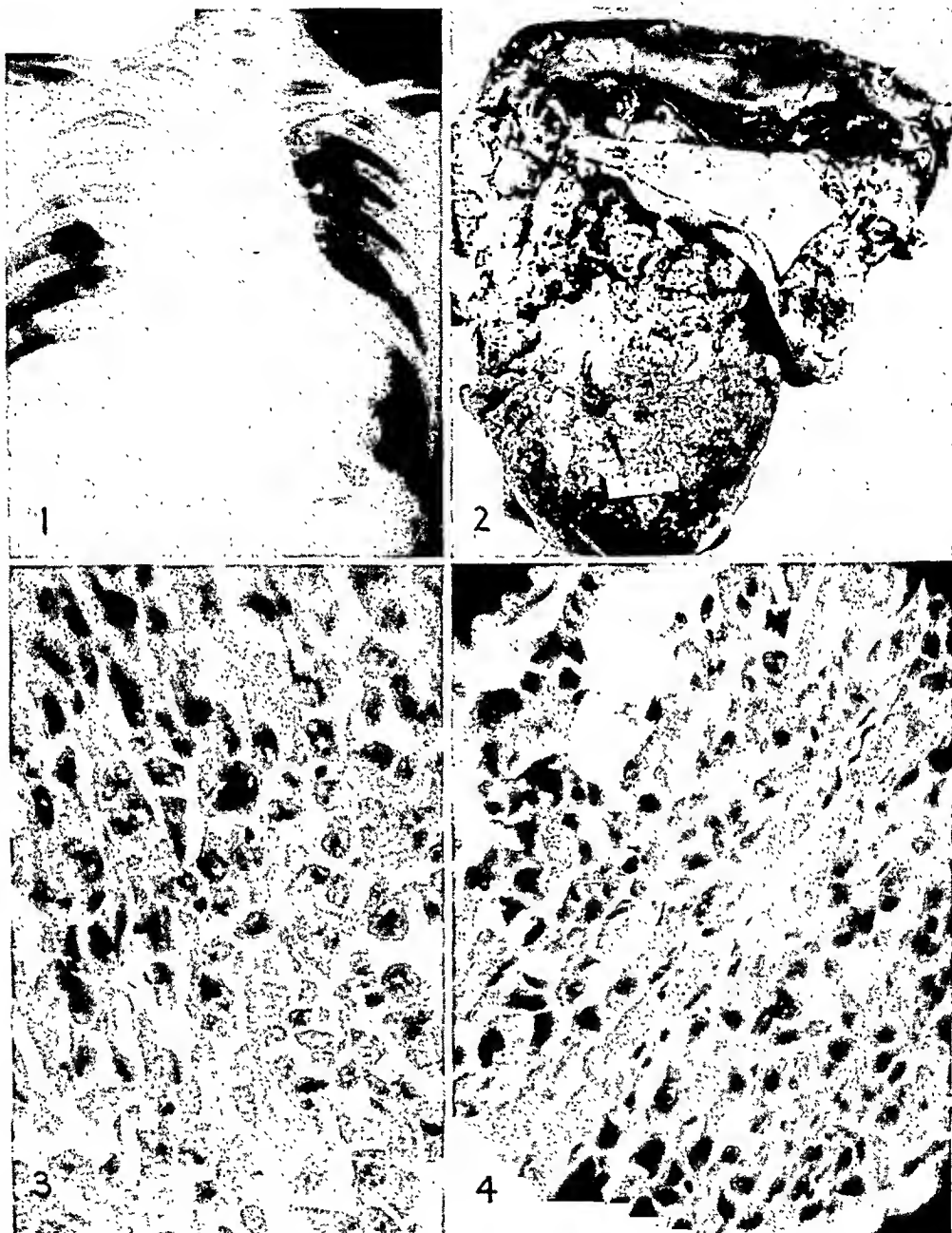
Fig. 1.—Roentgenogram of the chest. Note increased density in the right upper lung field and the right cardiophrenic angle.

Fig. 2.—Heart with the pericardium reflected upward, to show the extensive nodular involvement of the visceral and parietal pericardium.

Fig. 3.—Photomicrograph (high power) showing the pleomorphic cellular nature of the tumor of the pericardium.

Fig. 4.—Photomicrograph (high power) of the tumor's infiltration of the myocardium of the right ventricle.

fluid was dark amber in color and clear, and no growth occurred in culture mediums inoculated with it.

A provisional diagnosis of postpneumonic pleural effusion was made.

Cough was relieved with codeine. A transfusion of 500 cc. of whole blood was given. On the third day after admission the patient became suddenly dyspneic and died in twenty minutes.

Autopsy.—The principal findings were in the thorax and the abdomen. The right pleural space contained 1,500 cc. of dark amber, clear fluid; the left contained 150 cc. The pericardial sac was tense, distended, enlarged, dark blue and contained 1,000 cc. of bloody fluid. The surface of the left ventricle and the entire conus of the heart were studded with hard white tumor masses up to 3 cm. in diameter. Similar masses were attached to the internal surface of the parietal pericardium and to the pericardium over the great vessels (fig. 2). The heart weighed 450 Gm. The right ventricular surface was almost completely covered by coalescent tumor masses which invaded the heart muscle for 0.5 cm. The valve measurements were: mitral 90 mm., tricuspid 105 mm., aortic 75 mm. and pulmonary 78 mm. The right ventricular wall was 5 mm. thick and the left 15. The endocardium was unchanged. Atherosclerotic plaques were present in the root of the aorta. The coronary vessels were patent and not involved by the tumor.

The upper lobe of the right lung was partially collapsed, rubbery and dark red. The pleura over a part of the mediastinal surface of this lobe, 6 cm. in diameter, was studded with small white nodules similar to those in the pericardium. Extending from the pericardial cavity along the surface of the pulmonary artery were many small nodules 1 to 3 cm. in diameter. These masses pierced the pericardial sac and formed a large mass at the hilus, shutting off aeration of the upper lobe of the right lung and superficially infiltrating the adjacent lung tissue. The bronchi were carefully dissected, but no tumor could be found within the bronchi or the lung, although the upper right bronchus was markedly compressed. The upper lobe of the right lung was atelectatic. The remainder of the lung was not remarkable.

In the left lung there were many scattered nodules up to 2 cm. in diameter, but no areas of collapse were present. The left pleura was unchanged. The lungs together weighed 1,400 Gm. The tracheobronchial nodes were markedly enlarged; those in the right hilus contained tumor tissue as described.

The abdominal viscera were unchanged with the exception of the kidneys, which were studded throughout both cortices and medullas with small white firm tumors similar to those on the pericardium and elsewhere. The testicles, the prostate and the thyroid and adrenal glands were carefully examined for a possible primary tumor, but none was found.

Permission to examine the cranium was denied by the relatives.

The anatomic diagnosis was: primary mesothelioma of the pericardium extending locally to the myocardium, the hilus of the right lung and the pleural surface of the upper lobe of the right lung; atelectasis of the upper lobe of the right lung secondary to bronchial compression exerted by a hilar tumor mass; metastatic tumor of the left lung and both kidneys; hemopericardium; bilateral hydrothorax; generalized arteriosclerosis, grade I.

Microscopic studies were made on sections stained with hematoxylin-eosin, by Foot's method for reticulum, by the Azan method for connective tissue, and with Masson's trichrome and orcein-hematoxylin stains. Sections of the tumor and myocardium of the right ventricle revealed round to irregular tumor cells infiltrating the heart muscle, with destruction of the muscle fibers. The tumor was

highly cellular, and there was pronounced pleomorphism. The cells grew in cords with a slight suggestion of whorl formation, but no definite alveolar arrangement was seen (figs. 3 and 4). Many small blood vessels were present about the growing margin of the tumor. The cell nuclei were large with prominent nucleoli. The cytoplasm was acidophilic and finely granular. Mitoses were numerous. In sections through the hilar nodes there were areas of similar tumor cells infiltrating and destroying the lymphatic structure. Sections through tumor tissue of the pleura and underlying lung revealed masses of similar irregular tumor cells infiltrating the pleura and invading and compressing the pulmonary structure.

The supporting stroma consisted of a rather fine argentophil intercellular network. The nearby myocardium was infiltrated by polymorphonuclear leukocytes and occasional mononuclear wandering cells.

COMMENT

The diagnosis of pericardial mesothelioma must rest to a great extent on a process of elimination, in the absence of more reliable criteria. Robertson [10] cited a case in which the diagnosis was made, but subsequently the tumor was proved to be secondary to a small bronchogenic carcinoma. In our case we found no evidence that the pericardial tumor was metastatic. In sections of the bronchi taken in the area compressed by the tumor extension there was only slight atrophy of the mucosa. Cell arrangement and staining characteristics preclude the diagnosis of sarcoma of the pericardium.

McDonald [5] expressed a doubt that these tumors metastasize by the blood stream but acknowledged that lymphatic and direct extension are common. In our case, however, all forms of spread were encountered, as there were metastases in the left lung and the kidneys, direct invasion of the right hilar area and lung, and lymphatic spread to the right pleura.

SUMMARY ·

Mesothelioma of the pericardium is a tumor of great rarity. One case is added to the 22 recorded in the literature.

The diagnosis of mesothelioma can be made only after exclusion of all other possible primary foci.

10. Robertson, H. E.: J. Cancer Research **8**:317, 1924.

ADENOACANTHOMA OF THE ESOPHAGUS

A Report of One Case with Consideration of the Tumor's Resemblance to So-Called Salivary Gland Tumor

ELSIE McPEAK, M.D.

AND

WALTER L. ARONS, M.D.

BOSTON

CARCINOMA of the esophagus is usually epidermoid in type and in our experience this type shows keratinization in most cases. Other types, including adenocarcinoma, estimated as present in 10 per cent by Moore,[1] and an occasional embryonal carcinoma (Ewing[2]) are reported. Adenoacanthoma of the esophagus has received little attention. Kaufmann[3] mentioned that mucus was produced in secondary hepatic deposits of an acanthoma of the esophagus. Ewing[2] stated that the structure of carcinoma of the esophagus may vary in different portions of the tumor, with that in the nodes being either typical of acanthoma or more embryonal and atypical, but he made no reference to a combination of squamous cell carcinoma and adenocarcinoma. Recently, several observers (Wood,[4] Rabson[5] and Pasternack[6]) in reports of cases of adenoacanthoma of the gastrointestinal tract commented on this type as not unusual at the cardioesophageal junction, but only 1 case (Takagi[7]) of the 12 cases of adenoacanthoma of the stomach collected by Wood[4] could be considered as an instance of adenoacanthoma arising at the cardioesophageal junction.

In a survey of the literature we found reports of only 2 additional cases of adenoacanthoma involving the esophagus.[8] Both were Cabot

1. Moore, R. A.: Textbook of Pathology, Philadelphia, W. B. Saunders Company, 1944, p. 816.

2. Ewing, J.: Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders Company, 1940, pp. 605-606 and 936.

3. Kaufmann, E.: Pathology, Philadelphia, P. Blakiston's Son & Co., 1929, vol. 1, p. 641.

4. Wood, D. A.: Arch. Path. **36**:177, 1943.

5. Rabson, S. M.: Arch. Path. **21**:308, 1936.

6. Pasternack, J. G.: Am. J. Path. **11**:541, 1935.

7. Takagi, C.: Gann **31**:173, 1937.

8. (*a*) Cabot Case 21521, New England J. Med. **213**:1311, 1935. (*b*) Cabot Case 19122, ibid. **208**:652, 1933.

cases. In one [8a] only the esophagus was involved, while in the other [8b], the growth occurred at the cardioesophageal junction but the major portion of the tumor was in the stomach. Detailed descriptions and photographs of the tumor as observed in these 2 cases are not available, but it was said to be chiefly adenocarcinoma with limited areas of cornification.

REPORT OF A CASE

The patient was a 46 year old white man. The symptoms directly associated with the tumor were loss of 35 pounds (16 Kg.) and dysphagia over a period of eight months. In the last two months the patient had been unable to swallow solid food. Esophagoscopic examination revealed a tumor in the lower third of the esophagus. On exploration, metastases were found in the tracheal, aortic and retroperitoneal lymph nodes. A gastrostomy was performed, and the patient died two weeks later as a result of empyema of the right pleural cavity.

The anatomic diagnoses were: postoperative gastrostomy, recent; carcinoma of the esophagus with metastases in the liver, the kidney and lymph nodes (mediastinal, periaortic and celiac); pleural empyema, right; pericardial empyema, early; atelectasis of the right lung; pelvic peritonitis.

The primary tumor was entirely confined to the esophagus, being separated from the esophagocardiac junction by 2 cm. of uninvolved mucosa. It was an annular raised growth with well defined edges involving the esophagus over a portion measuring 10 cm. in length. The growth extended through all layers of the wall but did not infiltrate adjacent structures.

The metastases were not extensive. There were three nodules in the liver, two measuring 5 cm. each in diameter and one measuring 1 cm. in diameter. The nodule in the cortex of the left kidney measured 1 cm. in diameter. The lymph nodes along the trachea above the carina, along the abdominal aorta and around the celiac axis were firm and enlarged, and some of them were matted together.

Histologic examinations were made on multiple sections of both the primary tumor and the metastases involving the liver, lymph nodes and a kidney. The main part of the primary tumor and some areas of the metastases showed typical cornifying squamous cells with frequent pearl formation. The interesting and unexpected features of this tumor were revealed chiefly in the metastases, where a combination of adenocarcinoma, undifferentiated carcinoma and keratinizing carcinoma was observed (fig. 1). The particular pattern of this tumor is most commonly seen in certain tumors of the mouth and nasopharynx (Gates [9]).

In the primary tumor to a limited degree, and in a large part of the metastatic deposits, there were various-sized masses of rounded and polyhedral cells. In some instances these masses were sharply outlined by a peripheral layer of cylindric cells. Intimately intermingled with the cells of rounded or polyhedral shape were columnar cells, lined up in circular fashion to surround definite lumens that contained degenerated cells and nuclear debris (fig. 2). In the same masses of cells one noted at fairly frequent intervals single cells or small groups of cells which showed keratinization and in some areas suggested pearl formation (fig. 1). In some of these cell masses there was little or no evidence of keratinization, particularly in the renal metastatic deposit. In the primary

9. Gates, O.: Personal communication to the authors.
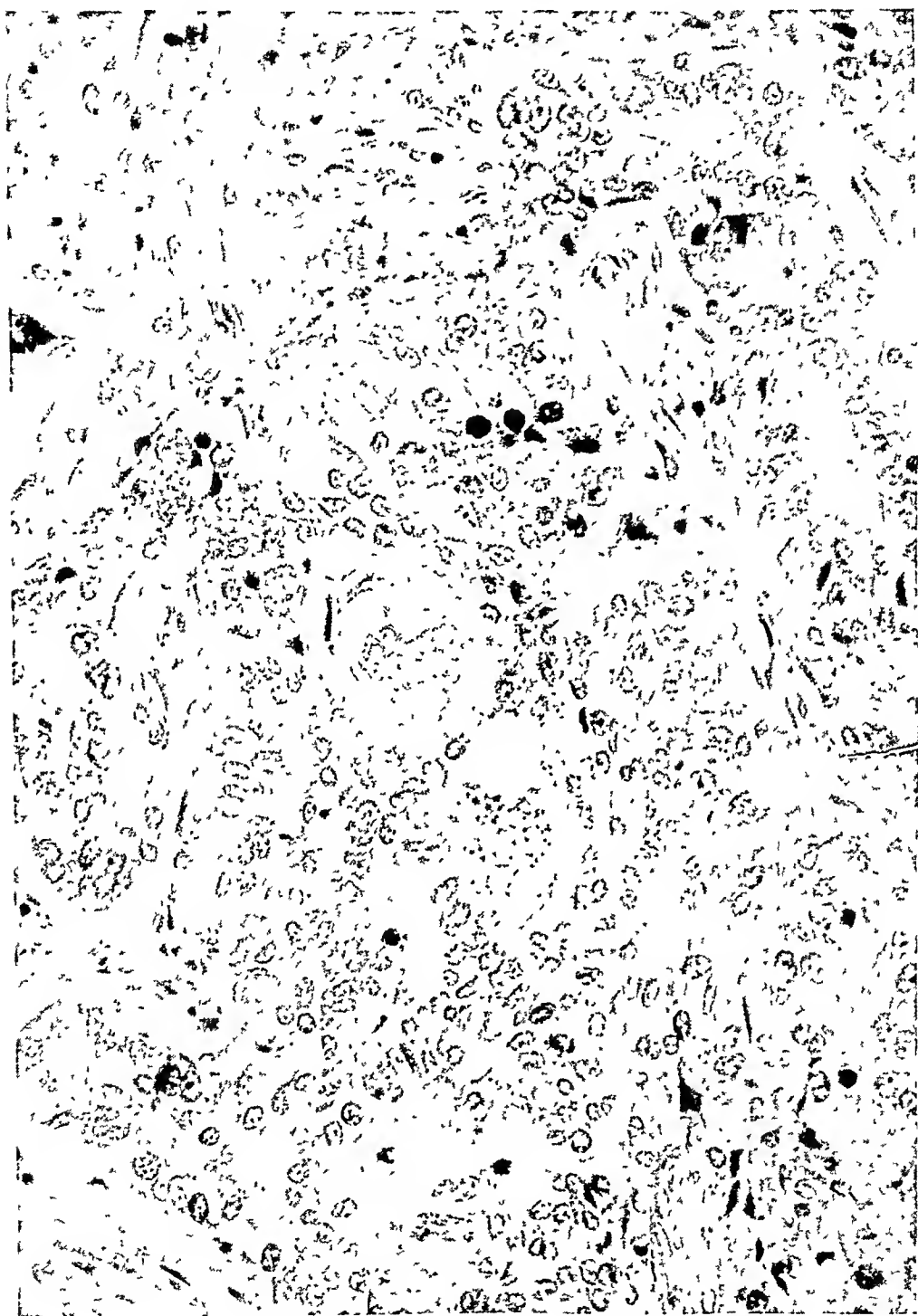
Fig. 1.—Combination of undifferentiated carcinoma and adenocarcinoma in which keratinized cells appear.
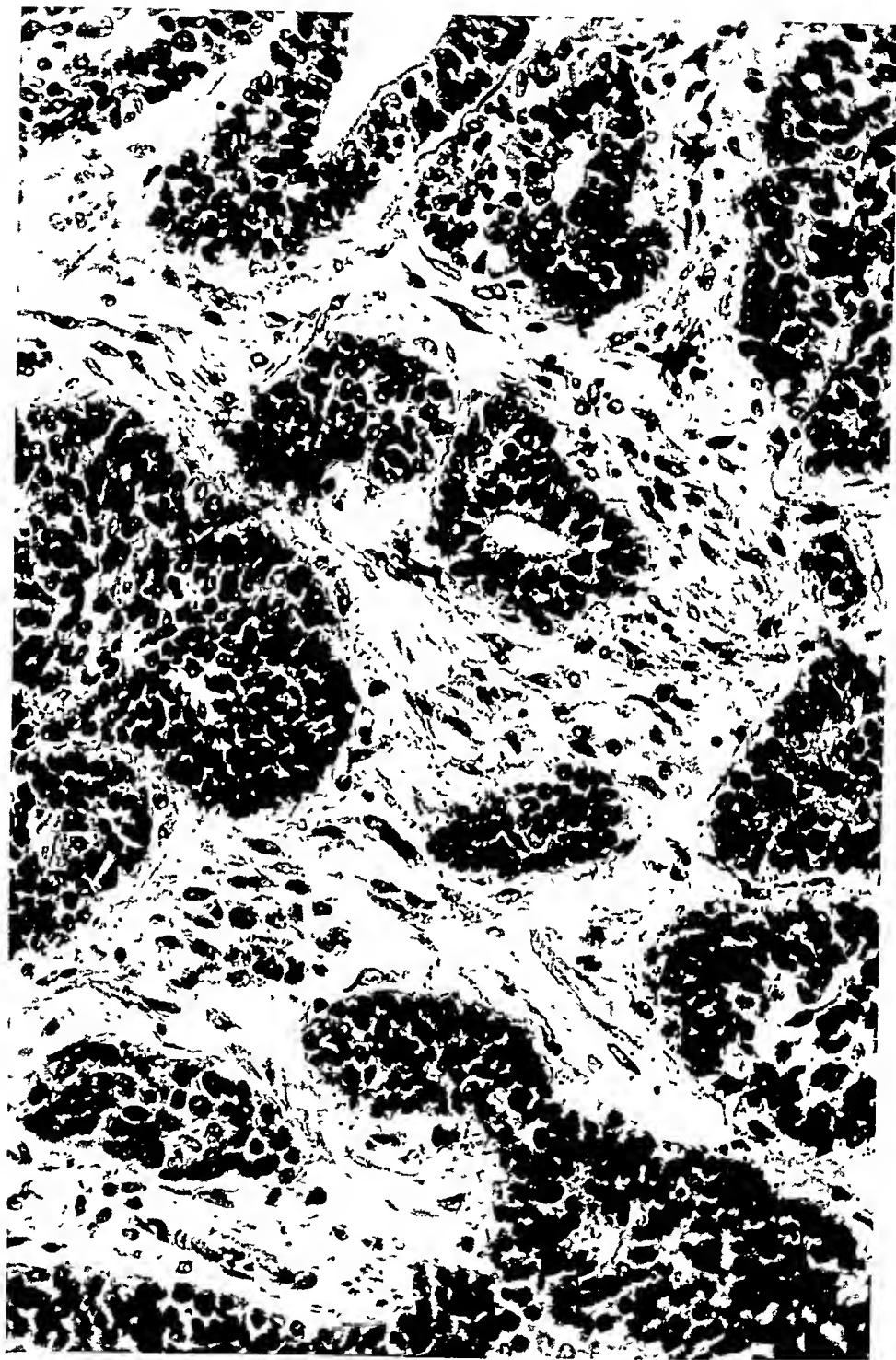
Fig. 2.—Undifferentiated masses of tumor cells with occasional gland formation.

tumor the other extreme was present, where only a few, poorly formed glands were seen near the mucosa of the surface and only a limited number of masses of undifferentiated cells appeared. The borders of the undifferentiated and the cylindric cells were poorly outlined. The eosin-stained cytoplasm of these cells varied from a light acidophilic, finely granular appearance to one which was almost clear. The nuclei were hyperchromatic and relatively large as compared with the cytoplasmic content. They varied only moderately in size or shape as contrasted with the nuclei of the differentiated squamous cells, which displayed marked polymorphism. Nucleoli were present in some nuclei but were not visible in others. Mitoses were extremely rare in the primary tumor but occurred more frequently in the metastatic deposits, up to 2 per high power field. All layers of the esophageal wall were infiltrated by the carcinoma. Necrosis occurred to a marked degree in some portions of the tumor, and central degeneration of the highly cornified carcinoma was common in both the primary tumor and the metastases. The invading tumor evoked a very slight fibroblastic reaction. Inflammatory cellular infiltration was variable, often slight, even in areas of necrosis, but occurred here and there, as evidenced by large groups of lymphocytes, plasma cells and polymorphonuclear and eosinophilic leukocytes.

COMMENT

Our attention was focused on certain special characteristics of this tumor that are usually found in the so-called salivary gland tumors. The tumor was different from the type of adenoacanthoma found elsewhere in the gastrointestinal tract as that usually appears. Comprehensive discussions of the histologic aspects and the histogenesis of that type of adenoacanthoma can be found in the literature.[10]

The importance of the mucous and serous glands of various body cavities as a source of carcinoma has only recently aroused general interest. In 1936 Ahlbom[11] gave a comprehensive account of the recorded tumors of this type, and his careful delineation of tumors of mucous-serous glands of the mouth was the most valuable and instructive contribution on the subject yet made. His work was followed by an equally useful description of similar tumors of the nasal cavities, accessory sinuses and nasopharynx, made by Ringertz[12] in 1938. Other workers, reporting single tumors and groups of tumors of this type, have confirmed and supplemented the observations of Ahlbom and Ringertz. In view of some reports of tumors of mucous and serous glands of the trachea and the bronchi (Ahlbom,[11] Sano and Meade[13]) the current classification of tumors of the lung would seem to need revision. As far as can be ascertained from the literature, no tumor of the mucous and the serous glands of the esophagus has been

10. Scheffler, M. M., and Falk, A. B.: Am. J. Cancer **38**:359, 1940. Ewing.[2] Wood.[4] Pasternack.[6]

11. Ahlbom, H. E.: Acta radiol., 1935, supp. 23.

12. Ringertz, N.: Acta oto-laryng., 1938, supp. 27.

13. Sano, M. E., and Meade, R., Jr.: Arch. Path. **43**:235, 1947.

reported, with exception of a tumor described by Kinoshita[14] which Ahlbom[11] stated had characteristics of the so-called mixed tumor of the parotid gland. In Ahlbom's[11] series there was no mucous or salivary gland tumor of the esophagus, but he stated that the rare reports of adenoma and basal cell carcinoma of the esophagus might relate to tumors belonging to this group. He reminded his readers that the wall of the esophagus contains mucous glands from which such tumors could arise.

In certain of its characteristics the tumor of the esophagus reported in the present paper was identical with portions of some tumors that are described as originating in the so-called salivary type of glands, i.e., mucous and serous glands (Ahlbom[11]; Ringertz[12]; Sheldon[15]; Quattlebaum, Dockerty and Mayo[16]). These characteristics of our tumor are those occasionally found in the areas of undifferentiated and gland-producing carcinoma in which there is keratinization of single or small nests of cells (fig. 1). It is not our intention to classify our specimen definitely with the so-called salivary gland tumors, since we have no proof that it originated from the glands of the esophagus, but it is possible that emphasis on the almost identical histologic patterns of tumors occurring in various locations may be important in the determination of the histogenesis of such tumors.

SUMMARY

A report of 1 case of adenoacanthoma of the esophagus was found in the literature. One additional case of adenoacanthoma of the esophagus is reported. The observation that certain portions of this tumor were similar to the so-called salivary gland tumors is discussed.

14. Kinoshita, M.: Schweiz. med. Wchnschr. **2**:156, 1921.

15. Sheldon, W. H.: Arch. Path. **35**:1, 1943.

16. Quattlebaum, F. W.; Dockerty, M. B., and Mayo, C. W.: Surg., Gynec. & Obst. **82**:342, 1946.

Laboratory Methods and Technical Notes

A MODIFICATION OF MAYER'S MUCICARMINE STAIN FOR MUCIN

MARY DRESBACH

Chief Technician, Section on Pathologic Anatomy, Mayo Clinic

ROCHESTER, MINN.

A MODIFICATION of Mayer's [1] mucicarmine stain for mucin has been employed routinely in staining for mucin in the laboratory of the section on pathologic anatomy of the Mayo Clinic for about two years. During this period more than 1,000 sections of tissues were prepared from current material as well as from stored older tissues which were to be used in special studies. The major advantage of the modification as compared with the original method lies in the marked contrast between the red staining of mucin and the staining of other tissues, which varies from black to blue to green (fig., upper part). With Mayer's original method, the background as well as the mucin tends to assume a pink color. Alcoholic fixation may be used but is not necessary, as most of the sections stained by the method herein reported were fixed in either 4 per cent solution of formaldehyde or Orth's fluid. The technic is rather simple and works well in the hands of technicians who have had little experience.

SOLUTIONS FOR FIXATION AND STAINING

Solutions for Fixation.—A 4 per cent solution of neutral solution of formaldehyde, Orth's solution or dehydrated or 95 per cent alcohol is used.

Solutions for Staining.—1. Weigert's iron-hematoxylin: (*a*) Subsolution A: hematoxylin, 1 Gm.; 95 per cent alcohol, 100 cc. Dissolve with the aid of gentle heat. (*b*) Subsolution B: Iron chloride, 29 per cent aqueous solution, 4 cc.; distilled water, 95 cc.; hydrochloric acid, 1 cc. Mix equal parts of subsolutions A and B. This mixture may be used for several days.

2. Mucicarmine: Carmine, alum lake, 1 Gm.; aluminum chloride, 0.5 Gm.; distilled water, 20 cc. Heat for two minutes or until deep red. Add 80 cc. of 50 per cent alcohol and filter. This solution should be no older than forty-eight hours. Filter again just before use.

3. Indigo Carmine: Two per cent aqueous solution of indigo carmine, 1 cc.; glacial acetic acid, 2 cc.; distilled water, 97 cc. Filter before use.

METHOD

1. Deparaffinize paraffin-embedded sections in the usual manner.

2. Treat tissue for thirty minutes in a freshly prepared solution made by adding

---

1. Mayer, P.: Ueber Schleimfärbung [1896], Neapel. Zool. Stat. Mitth. **12**:303, 1897.

glacial acetic acid to saturated aqueous solution of trinitrophenol (picric acid) to the concentration of 5 per cent.

3. Wash in running tap water until the tissue is no longer yellow.

4. Stain with Weigert's iron-hematoxylin stain for three minutes.

5. Remove excess stain by rinsing in tap water.

6. Differentiate in acid alcohol· (70 per cent alcohol to which hydrochloric acid has been added to the concentration of 1 per cent).

7. Rinse in tap water.

8. Place in a saturated aqueous solution of lithium carbonate (about 1.3 per cent) for five to ten seconds until the deep blue color is restored.

9. Wash five to ten minutes in running tap water.

10. Place in mucicarmine stain for thirty minutes.

11. Rinse off excess stain in tap water.

12. Counterstain lightly and quickly for about fifteen seconds with the solution of indigo carmine. The slides should be gently agitated while the tissue is being counterstained, so that the stain will be distributed evenly.

13. Rinse in tap water.

14. Dehydrate in 95 per cent methyl or ethyl alcohol and then in acetone or dehydrated alcohol.

15. Clear in xylene and mount in "clarite" or balsam.

RESULTS AND COMMENTS

Mucus in epithelium and connective tissue stains red; cartilaginous matrix, red; nuclei, black; cytoplasm and collagen, light blue; erythrocytes, green.

The mucus of normal gastric glands stains in a variable manner, as with earlier reported stains. Carcinoma which originates within the gastric mucosa, however, produces mucus which stains positively by this method.
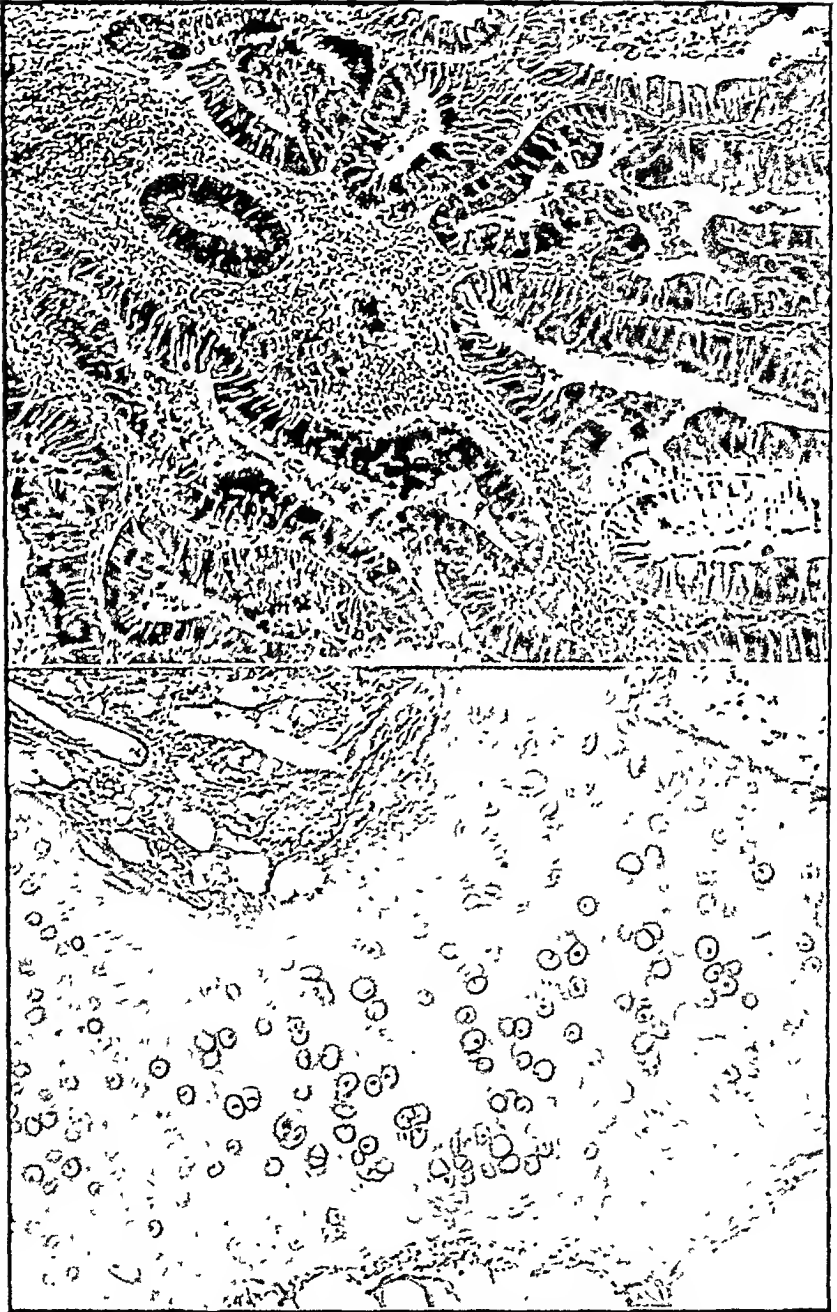
Tissues stained by the method described for mucus; the sites of mucus stain red.
Upper part: adenocarcinoma of the rectosigmoid (× 95). Lower part: chondroma
of the lung (× 70).

A NEW METHOD FOR BIOPSY OF THE LIVER

GUILLERMO HERRERA, M.D., and VICTORIANO PARDO, M.S.

HABANA, CUBA

THE GREAT INTEREST in hepatic biopsy by puncture [1] prompted us to construct a trocar which makes it possible, with the least amount of trauma and risk to the patient, to obtain sufficient tissue from the liver for a satisfactory histologic study.

The instrument which we present in this paper was constructed after considerable experimenting on cadavers. With it we are able to obtain a cylinder of tissue which enters the cannula after the removal of the stylet. The tissue is cut off by means of a thread at the end of the cannula.

DESCRIPTION OF THE INSTRUMENT

The trocar consists of a cannula with an attached wire and a stylet. The cannula varies from 0.78 to 2 mm. in diameter. At its distal end three holes are present, two smaller ones near the edge and one larger several millimeters farther back (*A* in figure).

The stylet, bevel shaped at its end, protrudes about 2 to 3 mm. beyond the end of the cannula. It is not completely cylindric; one side is flattened out to permit the passing of the two proximal ends of the wire between the cannula and the stylet As a result the circumference of the stylet forms a segment of a circle. Between the base of this segment and the cannula, space is provided for the wire. The distal end of the stylet is slightly thinner to provide space for the wire loop between the cannula and the stylet (*B* in figure).

A thin wire is used. We found it useful to employ metallic sutures 0.0031 inch (0.0775 mm.) in diameter.[2] The wire should be several inches longer than double the length of the trocar.

The two ends of the wire are introduced through the distal end of the cannula and each passes from the inside outward through one of the two small holes, leaving a loop of several centimeters outside the distal end. Both ends are then passed inward through the larger hole, to run through the entire length of the cannula and emerge at the proximal end. This last step may be facilitated by the use of a heavy wire which serves as a guide.

From the Clinical Service of Prof. C. F. Cardenás, University Hospital, University of Habana.

1. Iversen, P., and Roholm, K.: Acta med. Scandinav. **102**:1, 1939. Baron, E.: Arch. Int. Med. **63**:276, 1939. Tripoli, C. J., and Fader, D. E.: Am. J. Clin. Path. **11**:516, 1941. Dible, J H.; McMichael, J., and Sherlock, S. P. V.: Lancet **2**:402, 1943. Hoffbauer, F. W.; Evans, G. T., and Watson, C. J.: M. Clin. North America **29**:363, 1945. Gillman, T., and Gillman, J.: J. A. M. A. **129**: 12, 1945. Gillman, J., and Gillman, T.: Arch. Path. **4**:239, 1945.

2. The wire which we use is marketed under the name Surgaloy, size 40, by Davis & Geck, Inc., 57 Willoughby St., Brooklyn.

The next step is to pass the stylet through the cannula. This is facilitated by holding the wire at tension while the stylet is being passed. When this is accomplished the end of the stylet should pass through the loop of the wire at the distal end. The two free ends of the wire at the proximal end of the cannula are pulled taut. The distal end of the stylet is introduced into the distal opening of the cannula with the wire loop around the stylet (*C* in figure). The two proximal free ends of the wire are pulled taut, then bent forward and held in this position. The stylet is then taken out from the distal end of the cannula and introduced into the proximal end in such a way that the flat side of the stylet runs in ·the direction of the holes at the distal end of the cannula (*D* in figure).

This part of the procedure is somewhat difficult, but after some practice it can be done in from three to four minutes

When a trocar is less than a millimeter in diameter the metallic wire may interfere with the tissue entering the cannula A silk thread may be used instead.
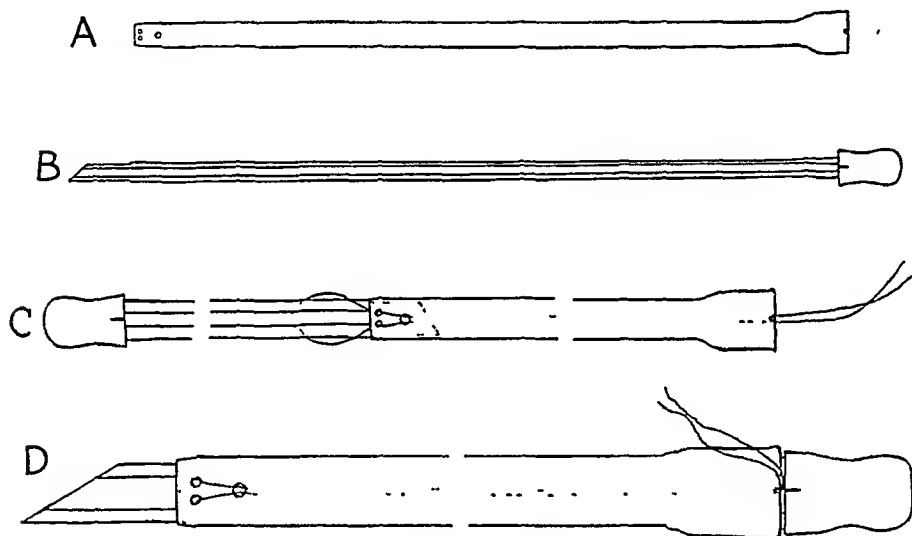
Trocar for biopsy of liver

TECHNIC OF BIOPSY

The portal of entry depends on the condition of the liver. When the liver extends 1 fingerbreadth or more below the right costal margin, we make the puncture through the abdominal wall just below the costal margin, in the midclavicular line If nodules are felt in the liver, the puncture is made at the level of a nodule.

If the liver is of normal size or slightly enlarged, an area of dulness is demonstrated in the right hypochondrium by percussion. The transpleurodiaphragmatic way is chosen at the level of the seventh or eighth intercostal space in the midclavicular or the anterior axillary line, depending on the size of the area of dulness.

If the liver is small or tilted backward by a distended stomach or intestine, the normal relations between the liver and the anterior part of the diaphragm are disturbed; it is then necessary to use the posterior transpleurodiaphragmatic route; the site of entry is the tenth or eleventh intercostal space about 8 to 10 cm laterally from the midspinal line.

When the anterior or the posterior transdiaphragmatic route is chosen, the trocar, after it has passed the thoracic wall in the costal interspace, is lowered

until it forms an angle of from 50 to 60 degrees with the wall of the chest, which makes its course perpendicular to the diaphragm. If the liver is small, this course frequently requires piercing the lung where the latter reaches the costodiaphrag-matic angle. This has not led to any complications, and piercing the lung is considered preferable to entering below the level of the liver.

The patient is prepared by oral administration of 0.1 to 0.2 grain of sodium seconal. · Morphine is not used.

After surgical preparation, anesthesia of the skin and the abdominal wall or of the intercostal space, as the case may be, is obtained by local infiltration. Then a small incision, 3 to 4 mm. long, is made through the skin. The trocar is intro-duced until it enters the liver. The stylet is then withdrawn and the cannula pushed for a distance of 2 to 3 cm. This will cause a block of liver tissue to enter the cannula as the latter penetrates the liver. The cannula is then held firmly in its position and the two free ends of the wire are pulled. In this manner the base of the cylinder of hepatic tissue is severed. The cannula is pulled out with the biopsy specimen in it. The wire is removed and the block of tissue is pushed out by the stylet. Still better is it to wash out the tissue with the help of a stream of fixative from a syringe.

COMMENT

Our experience of cadavers and of patients prompts us to claim that our method offers several advantages. The trocar causes little trauma, because of its small size and the rapidity of the procedure, thus reducing chances that the liver may be torn by respiratory movements of the diaphragm. We have always obtained sufficient tissue. The relatively long cylinder of tissue gives material from several levels.

We have used the method for biopsies of other organs. It may be of value for the diagnosis of pulmonary neoplasms, though our experi-ence of this type of lesion has been rather limited, and for the study of pneumonia. It is impossible to obtain a biopsy specimen of normal pulmonary tissue or even of tissue slightly consolidated, owing to elasticity. The lung firmly consolidated with pneumonia or tumor can always be easily punched. By obtaining specimens from different levels the trocar enables one to do a true selective biopsy.

We have also used the method on lymph nodes and on tumors of bone and of the breast, with good results.

SUMMARY

A method of obtaining liver for biopsy is described which makes it possible to obtain a cylinder of tissue as long as the distance covered by the trocar in the liver and of the same thickness as the diameter of the trocar. A wire loop at the end of the trocar is an essential feature of the instrument.

The trauma is minimal; abundant tissue is obtained in every case.

We have obtained biopsy specimens from 72 patients without a single hemorrhage or other complication.

STAINING TECHNIC FOR DIAGNOSTIC TISSUE SPREADS

HERMAN J. DICK, M.D.
SYRACUSE, N. Y.

A METHOD adapted from the staining of fecal smears [1] is described which is of use in staining material obtained in aspiration biopsy, body fluids and body secretions.[2] It was found to be superior to other methods, avoiding both the loss of cytologic detail coincident to drying [2d] and the usually unfamiliar color characteristics of the Papanicolaou stains.[3] It provides permanent preparations,[4] and it is offered as an adjunct to, but not as a substitute for, diagnosis by routine paraffin section.[2a,b]

Staining by the following method results in cytologic characteristics which are familiar to most pathologists and has proved satisfactory in most types of smear examination.

1. Fix spreads of fluid or other aspirated material in Schaudinn's solution for two to ten minutes at room temperature. Precise nuclear characteristics are lost if the smear is allowed to dry.[1]

2. Dehydrate in 70 per cent alcohol for five minutes.

3. Remove mercury in 70 per cent alcohol to which enough iodine has been added to give a port wine color—five minutes.

4. Rinse in 70 per cent alcohol.

5. Hydrate through 50 per cent alcohol and water.

6. Stain in Harris' hematoxylin for thirty seconds.

7. Rinse in tap water.

8. Blue in an alkaline solution (a pinch of sodium or lithium bicarbonate added to a staining jar of tap water) ·for one to two minutes.

9. Stain with 0.1 per cent eosin for two minutes.

10. Dehydrate in graded alcohol, clear in xylene and mount in balsam.

The entire procedure may be done in fifteen to twenty minutes. Spreading with a second slide as in making blood spreads is a convenient method, and it has been noted that individual cells are more easily identified in thin spreads. Spreads may be kept in the fixing solution or in 70 per cent alcohol for forty-eight hours without any harmful results. The fixative may be used until it becomes cloudy. It is impor-

From the Department of Pathology, Syracuse University College of Medicine.

1. Craig, C. F.: Laboratory Diagnosis of Protozoan Diseases, Philadelphia, Lea & Febiger, 1942.

2. (a) Foot, N. C.: Am. J. Path. **13**:1, 1937. (b) Graham, G. S.: ibid. **9**:701, 1933. (c) Herbut, P. A., and Clerf, L. H.: J. A. M. A. **130**:1006, 1946. (d) Stewart, F. W.: Am. J. Path. **9**:801, 1933.

3. (a) Gates, O., and Warren, S.: Am. J. Path. **21**:567, 1945. (b) Shorr, E.: Science **94**:545, 1941. (c) Herbut and Clerf.[2c]

4. Altgauzen, A. Y.: Am. Rev. Soviet Med. **4**:148, 1946.

tant that the spreads are not allowed to dry at any point of the procedure.[1] If drying occurs, it is then necessary to depend on size, variation and depth of diffuse coloring of nuclear material [2d] rather than on the sharp nuclear pattern with which pathologists have been familiar in the study of ordinary microscopic sections. Any protein present in the fluid examined is immediately precipitated. This does not interfere with the microscopic examination. Various modifications of the iron-hematoxylin stain and the 'phosphotungstic acid–hematoxylin stain [1] were not attempted since they are time consuming.

Since the interest was primarily in diagnosing cancer from spreads, nuclear detail [5] was considered most important. The chromosome pattern and the nucleoli retain the conformation and the color characteristics usually seen in material fixed in Zenker's solution. The cytoplasm is stained the usual pink with eosin, and basophilism of the cytoplasm when present is similar to that of good paraffin sections. The introduction of a new galaxy of colors, as in the various Papanicolaou modifications, is avoided.[6] The purpose of these various colors is apparently to permit determination of the origin of the cells or of their functional state. This is desirable for study of cyclic changes but unnecessary in the diagnosis of cancer. Cells as seen in spreads fixed and stained by this method have the same size as cells seen in sections. Individual cells or groups of cells can be identified as easily as in sections. The number of unidentifiable cells is kept to a minimum.

This method is best adapted to aspirated secretions [2] and exudates which are delivered immediately to the laboratory in a fluid state or are spread and fixed immediately at the time of biopsy. It is also well adapted to spreads or impressions of lymph nodes. Smears of peripheral blood and bone marrow are satisfactory in nuclear and cytoplasmic detail except that platelets are not stained. Polychromatic cytoplasm, granules and nucleoli are easily identified. It has been used with good success on material aspirated bronchoscopically from upper lobe bronchi.[2c]

SUMMARY

A more successful method is presented for the fixation and staining of spreads of fluids and exudates. This method emphasizes nuclear detail for identification of cells and minimizes the personal factor of "impression" in the study of smears or tissue fluid.

Note.—After this article was accepted for publication, references to a similar technic applied particularly to material aspirated from the bronchi were found in an article by L. S. Dudgeon and C. H. Wrigley (*J. Laryng. & Otol.* 50:752, 1935) and in one by J. Bamforth (*Thorax* 1:118, 1946). My results are exactly the same as those described and illustrated by Dudgeon and Wrigley.

In view of this there can be no question of priority, the principal results of my experience being (1) the superior fixation of cells in fluids and (2) the advantages of the familiar stains.

5. Altgauzen.[4] Gates and Warren.[3a] Graham.[2b] Herbut and Clerf.[2c]

6. Herbut and Clerf.[2c] Shorr.[3b]

General Reviews

MECHANISMS OF ABNORMAL DEVELOPMENT
I. Causes of Abnormal Development in the Embryo

PETER GRUENWALD, M.D.
NEW YORK

IN RECENT years investigation of abnormal development has gone far beyond the stage in which it consisted mainly of accumulations of descriptions of malformations and speculations as to the probable developmental stages of the anomalies under consideration. It is true that experiments were made long ago with the aim of producing malformations by means of various chemical and physical factors, but the agents were chosen arbitrarily and little of general teratologic significance was gained by this work. Only recent advances in developmental physiology and in genetics, as well as combined work in both fields, have yielded substantial and systematic knowledge of the mechanisms of abnormal development. These will be briefly discussed, and the morphology of malformations will be referred to only as far as is necessary in the course of this discussion of developmental mechanisms.

Many authors have in the past discussed the difficulties of defining malformations. Recent work has increased these difficulties, for it is now known that conditions which are generally recognized as malformations may be produced by a variety of agents which also cause diseases in postnatal life. It is certain that malformations are the result of abnormal development. Since any change of form is development, all abnormal forms might be considered as malformations. There is, for instance, no essential difference between disturbances of early embryonic development caused by chemical agents and, for instance, a tumor forming in the adult after treatment with a carcinogenic chemical. It is important to realize that teratology is a part of pathology not essentially different from the rest.

Knowledge gained in the study of developmental pathology of early stages may offer fundamental points of view for the study of the pathology of the adult, and vice versa. The age at which a disturbance takes place has often been considered as a criterion for malformation, and birth is set as the borderline. This cannot be strictly subscribed to, for identical malformations of teeth, for instance, may develop before and after birth, and typical inflammatory diseases which nobody would regard as malformations have been found in the embryo. No strict definition of a borderline between malformation and disease will be given here, not only because this is impossible but also because it is more profitable to stress the similarities than the differences between the two.

Several authors have discussed broad aspects of teratology in connection with their own descriptive, experimental-embryologic and genetic studies.[1] These reports are good examples of the scope and the success of modern teratology.

There are excellent recent accounts of the sciences which form the basis of developmental pathology. In the field of embryology, particularly in its physiologic aspects, the books of Spemann,[2] Weiss[3] and Needham[4] may be consulted. The last-mentioned author has gathered an impressive amount of the most modern information, including, among others, such borderline fields as embryonic metabolism

1. (a) Stockard, C. R.: Am. J. Anat. 28:116, 1921; (b) Am. Naturalist 58:24, 1924. (c) von Szily, A.: Ztschr. f. Anat. u. Entwcklngsgesch. 74:1, 1924. (d) Mohr, O. L.: Ztschr. f. indukt. Abstamm. u. Vererbgsl. 41:59, 1926; (e) Heredity and Disease, New York, W. W. Norton & Company, Inc., 1934. (f) Mangold, O.: Ergebn. d. Biol. 7:193, 1931. (g) Wright, S., in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1934, vol. 2, p. 137; (h) Physiol. Rev. 21:487, 1941. (i) Mann, I. C.: Developmental Anomalies of the Eye, London, Cambridge University Press, 1937. (j) Landauer, W.: Proc. Seventh Internat. Genet. Cong. Edinburgh, 1939; (k) Bulletin 236, Storrs Agricultural Experiment Station, 1941. (l) Murphy, D. P.: Congenital Malformations, Philadelphia, The Author, 1939. (m) Dunn, L. C., in Harvey Lectures, Baltimore, Williams & Wilkins Company, 1940, vol. 35, p. 135; (n) Growth (supp.) 5:147, 1941. (o) Potter, E. L., and Adair, F. L.: Fetal and Neonatal Death, Chicago, University of Chicago Press, 1940. (p) Snell, G. D.: Biology of the Laboratory Mouse, Philadelphia, The Blakiston Company, 1941. (q) Hamburger, V.: Biol. Symposia 6:311, 1942. (r) Grüneberg, H.: The Genetics of the Mouse, London, Cambridge University Press, 1943. (s) Gruenwald, P.: Am. J. Anat. 74:217, 1944.

2. Spemann, H.: Embryonic Development and Induction, New Haven, Yale University Press, 1938.

3. Weiss, P.: Principles of Development, New York, Henry Holt & Company, Inc., 1939.

4. Needham, J.: Biochemistry and Morphogenesis, London, Cambridge University Press, 1942.

and the cancer problem. In the field of genetics, there is, in addition to the standard texts, Grüneberg's [1r] book on the genetics of the mouse, which contains much of the best founded teratologic information now at hand.

The following example will be given to illustrate the changes that have been made in the approach to teratology during the past few decades. Cyclopia, one of the best studied single problems of this field, was first examined with regard to its morphologic aspects, and a series of degrees of the defect was established. It became apparent that the obvious ocular changes are but a part of a more extensive and complex defect, and it was logical to include lesser degrees of the malformation, in which the eyes were not quite fused (synophthalmia). The next step was to trace back the defects of the eyes and the brain to the stage of the flat, open neural plate. By a process comparable to showing a motion picture backward, the tissues of various parts of the eye and the brain were followed back to their position in the neural plate (to a stage in which they are not yet differentiated), and in this map of prospective retina, optic nerve, chiasma and other structures the cyclopic defects were outlined. It was thus shown that the missing structures are comprised in a wedge-shaped symmetric area.[5] The conclusion was reached that a wedge-shaped defect of the early embryo comprising as its most important part portions of the neural plate but also some of the underlying tissue causes cyclopia. Nothing could be said of the nature of this defect beyond the assumption that it never is an open cleft, as if tissue had been cut out, but is rather an absence of parts such that the bordering eye-forming tissue of the two sides develops as one fused mass from the very beginning. By this time experiments had been performed in which cyclopia was produced by treating embryos of various classes of vertebrates with chemicals.[6] The treatment was applied to the whole embryo at early stages, and the mechanism of action was not understood. Destruction of tissue in the aforementioned wedge-shaped area by mechanical means,[7] radiation [8] or electrocautery [8a] was shown to produce cyclopia, but it soon became clear that this was not the mechanism of spontaneous or of chemically produced cyclopia. A new con-

5. Fischel, A.: Arch. f. Entwcklngsmechn. d. Organ. **49**:383, 1921.

6. (a) Stockard, C. R.: J. Exper. Zool. **4**:165, 1907; (b) Arch. f. Entwcklngsmechn. d. Organ. **23**:249, 1907; (c) Anat. Rec. **3**:167, 1909. (d) McClendon, J. F.: Am. J. Physiol. **29**:289, 1912. (e) Werber, E. F.: J. Exper. Zool. **21**:485, 1916. (f) LePlat, G.: Arch. de biol. **30**:231, 1919.

7. Lewis, W. H.: Anat. Rec. **3**:175, 1909.

8. Wolff, E.: Arch. d'anat., d'histol. et d'embryol. (a) **18**:145, 1934; (b) **18**:229, 1934; (c) **22**:1, 1936.

cept stems from experiments made in amphibians. It had long been known that in the early embryo the mesoderm of the notochord and nearby areas acts on the covering ectoderm and induces it to form the neural plate. Adelmann [9] and Mangold [1f] were the first to show that median defects produced by excision in the mesoderm of the head caused, apparently also by way of induction, a change in the determinations within the neural plate which had been present in normal size and shape at the time of operation. This neural plate, without being directly affected by the experiment, as shown by controls, formed a cyclopic brain and eye. Thus, not the absence of tissue but abnormal determinations of its prospective parts caused cyclopia. Since induction and thus determination of one part by an adjacent tissue (in this case the mesoderm) are known to be mediated by chemicals released by the latter, one can understand that artificially introduced chemicals may interfere with this process and thus cause cyclopia and in a similar manner other malformations.

Several examples of hereditary median defects, including cyclopia, have been found.[11] This is just one of many instances in which similar if not identical malformations result from hereditary and environmental influences. An inkling of the direction in which the solution of this parallelism is to be sought is given by the increasing volume of evidence that genes act by determining the presence of enzymes and that mutations primarily affect the course of metabolism.[12] Thus the primitive idea of elimination of parts evidently missing in cyclopia has been supplanted by a physiologic concept which is far from complete, but stimulates progress. The formation of cyclopia will be discussed in more detail in part II of this review.

As was indicated by the introductory remarks and the preceding example, the present review will deal with the causes of abnormal development and with the mechanisms which follow the causes and produce the abnormal conditions commonly seen. It has already been stressed that there is no natural borderline between malformation and morbid disturbance of structure. The arbitrary limitation to embryonic disturbances which many writers have imposed on teratology is justified only so far as the principal structural pattern of the organism is determined and produced in the embryo. It is obvious that the most striking and far reaching structural abnormalities must develop in

9. Adelmann, H. B.: J. Exper. Zool. **57**:223, 1930.

10. Footnote deleted by the author.

11. Wright, S., and Eaton, O. N.: J. Agric. Research **26**:161, 1923. Wright, S.: Genetics **19**:471, 1934. Wright, S., and Wagner, K.: Am. J. Anat. **54**:383, 1934.

12. Beadle, J. W., in Harvey Lectures, Lancaster, Science Press, 1945, vol. 40, p. 179; Am. Scientist **34**:31, 1946.

these early stages. For this technical reason (and it should be realized that there is no other) the greater part of this review will also deal with the embryo. Since the close relationship of fetal diseases, such as infection, with malformations is becoming increasingly evident, fetal diseases will also be discussed. In all instances attention will be directed toward representative examples rather than complete listings of references.

The present review will appear in three parts. The first will deal with the causes of abnormal development, the second with the developmental mechanisms of embryonic malformations, and the third with examples of conditions in postnatal life which have been adequately analyzed along similar lines, including a brief discussion of the developmental aspects of tumors. Since the causes of most spontaneous developmental aberrations are not known, the second and third parts will have to treat of many conditions which have as yet not been traced to any of the causes described in the first part.

THE CAUSES OF ABNORMAL DEVELOPMENT

If one were to be exact in defining the cause of any particular malformation, it would be necessary to trace the abnormality back to the point where an external force produced the first aberration of any kind from the normal condition. In the case of a hereditary abnormality this means going back through all generations carrying the abnormal gene, to find the external cause of the mutation. This is obviously impossible with the so-called spontaneous mutations, even though one may assume that they, just as those induced in the laboratory, have external causes and are therefore not truly spontaneous in the last analysis. Even in the case of a nonhereditary malformation, in which the cause must have acted on the individual itself, investigators are usually unable to find that cause later on unless they have produced the malformation in the laboratory. This illustrates the value of the experimental production of developmental disturbances, as they are the only ones in which the mechanism can be discovered with any degree of dependability. They are models which show how similar malformations of unknown causes may have developed. However, greatest caution must be observed in applying experimental results to malformations found in nature. The previously quoted example of cyclopia shows that many different causes and even entirely different developmental mechanisms may produce very similar end results. Many more examples of this will be quoted in the following pages.

The causes of abnormal development to be discussed here may be classified as follows:

Genetic causes (including influences on the germ cells probably affecting the genotype):

Mutations with unknown causes ("spontaneous")

Mutations induced by $\begin{cases} \text{radiation} \\ \text{chemical treatment} \end{cases}$

Hybridization

Overripeness of the egg cell ∕

Somatic mutations

Agents affecting the phenotype without effect on the genotype:

Mechanical agents

Radiations

Chemical influences $\begin{cases} \text{addition of substances} \\ \text{deficiencies} \end{cases}$

Temperature changes

Infections

The spatial relation of the causative agent to the affected part has been discussed by Gruenwald.[18] Three possibilities exist for the location of the agent: It may be (*a*) within the primarily affected part, (*b*) within the organism but outside the primarily affected part or (*c*) outside the organism.

The first-mentioned relationship prevails in genetically determined malformations and includes also normal properties of the part in question, which make it more susceptible to extrinsic influences. The second possibility exists if abnormal, or rarely normal, conditions elsewhere in the body cause or favor maldevelopment. The third possibility includes the chemical, physical and infectious agents to be discussed in later sections. It should be noted that this classification includes, in addition to frank teratogenic agents, conditions which are not harmful in themselves but which predispose a given part to abnormal development. No sharp distinction of these two types of agents or conditions is possible. In some cases each is ineffective without the other.[18]

GENETIC CAUSES OF MALDEVELOPMENT

In the case of a typical hereditary malformation an abnormal gene appears by mutation with or without an apparent cause. This gene, in homozygous or heterozygous [13] condition, alone or in combination with other genes or with extrinsic agents, causes abnormal development. The long history of attempts to modify the genetic constitution

---

13. In the homozygous condition the corresponding places in the two sets of chromosomes of each body cell are occupied by genes of the same quality; in the heterozygous state these two places are occupied by alleles which affect the same traits in different manners, and of which either the normal or the abnormal one may dominate over the other.

will not be reviewed. The problem of the inheritance of acquired properties is still unsolved, as is the related problem of the manner in which species are modified in nature and new species formed. There is, however, unquestionable evidence of the existence of agents by which mutations and consecutive hereditary malformations can be produced. It is generally acknowledged that the genes are located in the chromosomes, which undergo regular and complicated changes during mitotic division of the cell. It is therefore not surprising that agents which are known to have a profound influence on mitosis, namely, certain radiations,[14] are also most potent in the experimental production of mutations. Ultraviolet and roentgen rays have been used extensively in the study of mutations of such primitive organisms as bacteria and fungi,[15] and much of the recent fundamental knowledge of the manner in which genes act on metabolism is derived from this work.

An interesting group of investigations made in amphibians will be mentioned here because the effect is transmitted to the embryo by one of the germ cells, even though it is not certain that the effect is of a genetic nature. Beginning many years ago, numerous workers[16] have irradiated male or female germ cells previous to fertilization and have reported early death of the embryo or, if suitable doses were used, various severe malformations. Among these are abnormalities of gastrulation and defects of the brain and eyes. It was at first surprising that severe irradiation of spermia is followed by normal development. This happens because the chromatin of the spermia is so severely damaged that it does not take part in further development. However, the spermia are still motile and stimulate the egg cells to develop, in a parthenogenetic manner as far as the chromatin is concerned. Accordingly, the embryo has the haploid number of chromosomes, derived entirely from the egg cell.[17] Henshaw[18] confirmed these results and found, in addition to deformities of organs, anaplastic papillomatous growth of the ectoderm, similar to that resulting from development of overripe eggs (see page 407) and other experimental procedures.

The possibility of producing mutations in mammals by the action of roentgen rays has been examined in great detail. Irradiated male mice

14. Politzer, G., in Chambers, R., and others: Protoplasma-Monographien, Berlin, Verlagsbuchhandlung Gebrüder Borntraeger, 1934, vol. 7.

15. Gray, C. H., and Tatum, E. L.: Proc. Nat. Acad. Sc. **30**:404, 1944. Beadle.[12]

16. (a) McGregor, J. H.: Science **27**:445, 1908. (b) Bardeen, C. R.: Am. J. Anat. **11**:419, 1911. (c) Hertwig, O.: Arch. f. mikr. Anat. **77**:1, 1911; (d) **77**:165, 1911; (e) (supp.) Anat. Anz. **54**:94, 1921. (f) Rugh, R.: Proc. Am. Philos. Soc. **81**:447, 1939.

17. Hertwig.[16e] Rugh.[16f]

18. Henshaw, P. S.: J. Nat. Cancer Inst. **3**:409, 1943.

had offspring with hereditary malformations of brain, eyes, face, extremities and urogenital tract. These abnormalities have been followed through many generations.[19] Their embryologic aspects will be described in part II. The causal relationship between irradiation and the origin of this mutation has recently been questioned,[1r] but there are other instances, involving mostly malformations of the brain, in which the hypothesis that the mutation is caused by roentgen rays is more probably true.[20]

This work has raised the question whether or not therapeutic irradiation of the ovaries or the testes of man can be the cause of abnormal offspring by producing mutations. This has been discussed extensively by geneticists as well as by clinicians. It is obvious that damage to the offspring by mutations induced in the parental germ cells must be clearly distinguished from noninherited damage due to the embryos having been irradiated in utero. The former may not be apparent until two or more generations hence. The latter, which is definitely known to produce malformations, will be referred to in a subsequent section.

There is no direct proof that irradiation of the maternal ovaries preceding conception produces malformations in the offspring. A review of 265 cases [21] showed 5 per cent defective children, but it was pointed out that these small numbers of defects not conforming to any definite pattern of maldevelopment may well be due to other influences of the environment of the fetus, since the mothers had some pathologic conditions requiring roentgen therapy. Another writer [22] asserts with questionable logic that there is no danger for pregnancies following therapeutic irradiation, even though he has found an increased incidence of abortions as well as of retarded development of the children in later years. The possibility of roentgen ray damage of the internal genital organs of the mother with the result that the development of the ovum may be interfered with must also be taken into consideration.[23] Several authors have reviewed animal experiments, some of which were referred to in foregoing paragraphs, and have concluded that damage of the genetic constitution of the immature egg cells of the ovary is improbable if not impossible.[24] Statistical evaluation of the probability of mutations led to similar conclusions.[25] However, several authors

19. Little, C. C., and Bagg, H. J.: J. Exper. Zool. **41**:45, 1924. Little, C. C.: Am. Naturalist **65**:370, 1931.

20. (*a*) Snell, G. D.; Bodemann, E., and Hollander, W.: J. Exper. Zool. **67**:93, 1934. (*b*) Snell, G. D., and Picken, D. I.: J. Genet. **31**:213, 1935. (*c*) Snell, G. D.: Radiology **36**:189, 1941.

21. Murphy, D. P.: Surg., Gynec. & Obst. **48**:766, 1929.

22. Werner, P.: München. med. Wchnschr. **68**:767, 1921.

23. Borak, J.: Arch. f. Gynäk. **147**:304, 1931.

24. Murphy, D. P.: Surg., Gynec. & Obst. **47**:201, 1928. Borak.[22]

25. Peller, S.: Arch. f. Gynäk. **147**:360, 1931.

recommend caution and protection of the gonads from unnecessary irradiation even though the probability of ill effects may be small.[26] Schubert[26d] points out in this connection that in the production of mutations only the total dose counts, regardless of the size and intervals of the single doses, whereas Müller[26b] gives a maximum daily dose which he considers safe. No objection to diagnostic doses as used for roentgenograms has so far been raised. Snell[20c] holds that while gene mutations (affecting single genes) are not to be expected, chromosome mutations, such as translocation of larger portions of a chromosome, are far more probable. He agrees with other writers that the only proved instance of damage of the offspring due to irradiation of the germ cells is that of damage caused by treatment of mature sperm cells. It thus appears to be the opinion of most authors that while maldevelopment has been proved to occur only when mature sperm cells have been irradiated shortly before fertilization, greatest caution should be exerted and the gonads of persons in the reproductive age protected from roentgen rays. In the male the typical irradiation effect consists of a transient period of sterility, which does not immediately follow the treatment. Only fertilization occurring during the period between treatment and this temporary sterility is definitely known to produce defective offspring.[27]

Little conclusive work has been reported on hereditary changes produced by methods other than irradiation. There are a few reports on the offspring of animals treated with chemical agents. They are important from the medical point of view because they concern substances to which human beings may be exposed. Stockard and his coworkers[28] describe in the descendants of alcoholized guinea-pigs a hereditary inferiority resulting in a reduced number of offspring and early death of many young. Pearl[29] exposed chickens to vapors of ethyl alcohol, methyl alcohol or ether and found a reduction in the number of fertile eggs but a lower prenatal and postnatal mortality of the chickens derived from fertile eggs as compared with his controls. There was also a higher mean adult body weight, and no increase in the incidence of malformations. In order to explain the discrepancy of his own results and those of Stockard and others not mentioned

26. (a) Murphy, D. P., and Goldstein, L.: Am. J. Roentgenol. **22**:207, 1929. (b) Müller, J. H.: Monatschr. f. Geburtsh. u. Gynäk. **109**:105, 1939. (c) Muller, H. J.: Nature, London **147**:718, 1941. (d) Schubert, G.: Röntgenpraxis **13**:1, 1941.

27. Borak.[23] Snell.[20c]

28. Stockard, C. R., and Craig, D. M.: Arch. f. Entwcklngsmechn. d. Organ. **35**:569, 1912. Stockard, C. R., and Papanicolaou, G. H.: Am. Naturalist **50**:144, 1916.

29. Pearl, R.: J. Exper. Zool. **22**:241, 1917.

here, Pearl proposes the following hypothesis: The germ cells of a given species differ in their capacity to produce normal, sturdy offspring and also in their resistance to damage caused by alcohol or other means. These two properties are coupled so that in some species and under certain conditions alcohol will kill those' cells which would otherwise produce weak offspring and leave the others unharmed, and in other species alcohol will also damage the remaining cells. The former would explain Pearl's own results with chickens, and the latter, Stockard's findings in guinea pigs. In this manner the seemingly conflicting results may be explained. In a more recent review of the subject, P. Hertwig [30] cites many articles accepting or denying an effect of alcohol on the progeny, and concludes that the final answer has yet to be found.

Landauer [31] published a preliminary report on the offspring of cocks treated with thallium. There was a high mortality during a narrowly limited period of time within the first two weeks after hatching, which varied slightly with the intensity of the fathers' treatment. These experiments have not been continued on a satisfactory scale, and Landauer himself [32] considers his results as significant but not conclusive.

The offspring of guinea pigs affected by lead poisoning have been examined.[33] They showed a reduced birth weight, an increased death rate during the first postnatal week and general retardation of development. When the lead treatment ends, the gonads recover, and the new progeny is normal. There is no mention of offspring of the retarded young.

Somewhat doubtful is the interpretation of hereditary malformations of the eye, of the progeny of rabbits treated with the serum of fowls immunized to lens tissue.[34] The malformations in later generations include not only opacity of the lens but also microphthalmia with defects of other parts of the eyes. In the progeny of chickens treated with naphthalene or alcohol, cataract and coloboma have been described.[35]

The effect of overripeness of the egg cell on development should be mentioned at this point, even though there is no conclusive evidence that it is due to changes in the genetic structure. However, the effect is transmitted to the embryo by one of the germ cells, just as were the radiation effects in some of the aforementioned experiments on germ cells. Overripeness of the egg at the time of fertilization has been studied in several classes of vertebrates and found to be associated

30. Hertwig, P.: Jahresk. f. ärztl. Fortbild. **26**:50, 1935.
31. Landauer, W.: Arch. f. Gewerbepath. u. Gewerbehyg. **1**:791, 1931.
32. Landauer, W.: Personal communication to the author.
33. Weller, C. V.: J. M. Research **28**:271, 1915.
34. Guyer, M. F., and Smith, E. A.: J. Exper. Zool. **31**:171, 1920. Davis, F. A.: Tr. Ophth. Soc. U. Kingdom (pt. 2) **45**:555, 1925.
35. Kusagawa, S.: Arch. f. Ophth. **118**:401, 1927.

with a reduction in the number of offspring and a variety of developmental disturbances. In the trout various malformations, including double monsters, were found, as well as a change of the sex ratio in favor of males.[36] The latter has been explained by transformation of some genetic females, due to a somatic cause. Similar sex changes in frogs developing from overripe eggs were studied in detail by Witschi.[37] Various malformations, including duplicitas, were also found in them.[38] As a severe form of this disturbance, entirely unorganized growth was observed which, when transplanted to normal tadpoles, grew in the manner of a cancer.[39] Other investigators do not assume a true cancerous nature, pointing out that only in weak hosts aggressive growth occurs.[40] In the guinea pig and the rat a reduced number of embryos, frequent death in utero, and malformations follow delayed insemination.[41] No normal development occurs in the guinea pig if fertilization is delayed more than twenty hours after ovulation, and no development at all if the interval is longer than thirty-two hours. In the rat the limit for normal development of at least part of the embryos is fertilization ten hours after ovulation. In most mammals overripeness of the egg is normally prevented, as the female admits the male only during estrus, i. e., at the optimal time. Nothing is known to suggest the occurrence of malformations due to overripeness in man, in whom no such protective mechanism exists.

Another probable instance of genetically controlled malformations has been reported but not sufficiently worked out. Loeb [42] and Newman [43] found that eggs of the fish Fundulus when fertilized with spermia of other fish species yield large numbers of various malformations. These malformations resemble those Werber obtained by chemical treatment of normally fertilized eggs (page 417) and include, among others, double monsters, cyclopia and various other defects of large parts of the body, leaving in some instances only isolated eyes or hearts. Loeb is inclined to assume that in these cases the eggs develop parthenogenetically as far as their sets of chromosomes are concerned—in other words, that the chromosomes of the spermia are

36. Mršić, W.: Arch. f. mikr. Anat. u. Entwcklngsmechn. **98**:129, 1923.

37. Witschi, E.: Arch. f. Entwcklngsmechn. d. Organ. **102**:168, 1924

38. Witschi, E.: Proc. Soc. Exper. Biol. & Med. **31**:419, 1934. Zimmerman, L., and Rugh, R.: J. Morphol. **68**:329, 1941.

39. Witschi, E.: Proc. Soc. Exper. Biol. & Med. **27**:475, 1930.

40. Briggs, R. W.: Anat. Rec. **81**:121, 1941. Briggs, R. W., and Berrill, N. J.: Growth **5**:273, 1941.

41. Blandau, R., and Young, W.: Am. J. Anat. **64**:303, 1939. Blandau, R. J., and Jordan, E. S.: ibid. **68**:275, 1941.

42. Loeb, J.: J. Morphol. **23**:1, 1912; Biol. Bull. **29**:50, 1915.

43. Newman, H. H.: Biol. Bull. **32**:306, 1917.

lost. In support of this he shows that similar malformations can be obtained from normally fertilized eggs by various external influences. However, it was mentioned in a preceding paragraph that if spermia damaged by various agents [16] or derived from a different species [44] merely induce parthenogenesis, the incidence of malformations is not unusually high. Further investigation of this problem should be of great interest.

Numerous hereditary malformations of unknown cause have been found and extensively studied in the breeding of laboratory and domestic animals. Several of these have been investigated embryologically, with highly gratifying results, as will be reported in part II.

The details of the genetic mechanisms involved will not be discussed here, as such an excursion would lead far into the field of genetics. Several reviews of various aspects of the subject are available.[45] Many of the well studied hereditary malformations are transmitted by single factors, the abnormal trait being either dominant or recessive, or having a different expression in homozygous and heterozygous form. A large number of hereditary traits are known which in the homozygous condition interfere with life beyond embryonic or early postnatal periods, and the genes producing these severe malformations in homozygous individuals are called lethal factors. The embryos carrying this condition and destined to die before reaching maturity should, according to Cairns,[46] be called prothanic rather than lethal. The best studied example is the Creeper chick which will be discussed later in more detail.

The expression of single genetic factors may be modified by other genetic [47] or by environmental [48] factors. Some of these modifications increase the severity of the defect. There is, for examples a cumulation of severity of malformations if Creeper chick embryos develop under the influence of Selenium intoxication.[49] Other instances are known in which the embryo may benefit from the action of additional influences. Remarkable among these are: changed uterine environment in an early lethal malformation of the mouse,[50] temporary lowering of

44. Rugh, R., and Exner, F.: Proc. Am. Philos. Soc. **83**:607, 1940.

45. (a) Mohr.[1e] (b) Snyder, L. H.: Medical Genetics, Durham, N. C., Duke University Press, 1941; (c) Am. Naturalist **76**:129, 1942. (d) Baur, E.; Fischer, E., and Lenz, F.: Human Heredity, New York, The Macmillan Company, 1931.

46. Cairns, J. M.: J. Exper. Zool. **88**:481, 1941.

47. (a) Reed, S. C., and Snell, G. D.: Anat. Rec. **51**:43, 1931. (b) Reed, S. C.: Genetics **21**:361, 1936. (c) Dunn.[1n] (d) Grüneberg.[1r] (e) Dunn, L. C., and Gluecksohn-Schoenheimer, S.: Proc. Nat. Acad. Sc. **31**:82, 1945.

48. Dunn, L. C.; Gluecksohn-Schoenheimer, S.; Curtis, M. R., and Dunning. W. F.: J. Hered. **33**:65, 1942. Dunn.[1n] Grüneberg.[1r]

49. Landauer, W.: J. Exper. Zool. **83**:431, 1940.

50. Robertson, G. G.: Genetics **27**:166, 1942.

the incubation temperature[51] and possibly also increased oxygen supply[46] in the case of the homozygous Creeper chick. Similarly, the development of hereditary polydactyly in chickens may be suppressed by lowering the temperature at incubation.[52] However, no influence of lowered temperature was found in several other hereditary malformations of the chick.[53]

Another noteworthy fact is that identical or very similar abnormalities are produced by mutations occurring at different points of the pattern of genes.[54] Finally one should mention the peculiar effect which results if an abnormal genetic factor is located in the sex chromosome. The possibilities for the expression of this factor will then differ according to sex, and this has been termed sex-linked inheritance. Many examples have been described in human and animal pathology—for example, hemophilia and certain forms of color blindness. For the details of this hereditary mechanism textbooks of genetics should be consulted.

The mechanism of gene action as observed during the development of an individual and throughout life is not well understood. In some instances it appears that the presence of enzymes is governed by genes, and genetically controlled enzyme deficiencies are followed by accumulation or excretion of intermediary products which cannot be metabolized. This has been studied in detail in fungi, and similar conditions seem to be present in human alkaptonuria and phenylketonuria,[12] lipid storage diseases[55] and glycogen storage disease.[56] Structural abnormalities are not readily explained by this mechanism. Only in the case of gargoylism ·(lipochondrodystrophy) it has been suggested that the disturbances in skeletal development are caused by accumulations of an as yet unidentified substance in the cartilage cells.[57] In many malformations the gene action is supposed to affect the rate of metabolism and of growth at a definite stage of development,[4] interfering primarily with those parts which grow most actively at that moment.[1a] The problem of multiple ("pleiotropic") gene action will be discussed in part II of this review.

In the preceding pages only those malformations have been considered which are caused by the action of abnormal genes. There

51. Landauer, W.: Science **100**:553, 1944.

52. Sturkie, P. D.: Genetics **27**:172, 1942; J. Exper. Zool. **93**:325, 1943.

53. Sturkie, P. D.: Am. Naturalist **79**:286, 1945.

54. Dunn, L. C., and Gluecksohn-Schoenheimer, S.: Proc. Nat. Acad. Sc. **30**:173, 1944.

55. Sobotka, H.; Glick, D.; Reiner, M., and Tuchman, L.: Biochem. J. **27**:203, 1933. Sobotka, H.: J. Mt. Sinai Hosp. **9**:795, 1942.

56. Bridge, E. M., and Holt, L. E.: J. Pediat. **27**:299, 1945.

57. Schmidt, M. B.: Centralbl. f. allg. Path. u. path. Anat. **79**:113, 1942.

are, however, a few conditions in which abnormalities develop through unfavorable constellations of genes which are normal in themselves. This is the case in certain instances of genetic intersexuality.[58] While the intersexuality which occurs in some breeds can be accounted for by gene mutations,[59] that in others has been produced by abnormal combinations of normal genes. There are not only polyploid individuals (in lower species of animals) in which abnormal numbers and proportions of normal male and female determining factors can produce abnormal sex development by their interaction, but also diploid individuals in which intersexuality has been found to be due to a faulty quantitative relationship of sex determining factors.[60] This has been studied extensively by Goldschmidt [61] in crosses of various local races of the gypsy moth, Lymantria dispar. It was found that some of these races differ in the quantitative effect of their male and female determining factors. Within each race the strengths of these factors are so matched that a sufficient preponderance of one sex is present to assure normal sex development. In crosses, however, this preponderance may be insufficient if male and female factors come from races with factors of different strength. Thus, in an individual which, according to the number of its sex chromosomes, should be female, the male factors may be relatively too strong, or vice versa, and intersexuality will result. The two opposing views on the manner in which intersexual traits develop in these individuals during embryonic life will be discussed in part II. It must be emphasized that in most species the strengths of the sex factors do not vary among races and, consequently, the overwhelming majority of interracial crosses will not result in intersexuality. There are few examples of intersexuality appearing in interracial crosses in vertebrates, and these have not been fully analyzed from the genetic angle. If this analysis could be undertaken, these instances might well turn out to be due to mutations rather than to an abnormal combination of normal factors as was just discussed.

In another instance of developmental disturbances caused by normal genes the mechanism is entirely different. It is not an interaction of ill matched genes during the development of the traits which they

58. The term "intersexuality" was used by Goldschmidt [61] to indicate those abnormal sexual conditions which result from sex reversal during development (see part II of this review). This theory is not generally accepted. The term is now frequently used to indicate sexual intergrades of various kinds.

59. (a) Bonnevie, K.: Arch. f. Entwcklngsmechn. d. Organ. **106**:611, 1925. (b) Riddle, O.; Dunham, H. H., and Schooley, J. P.: Genetics **27**:165, 1942. (c) Eaton, O. N.: ibid. **30**:51, 1945.

60. Bridges, C. B., in Allen, Danforth and Doisy,[64] p. 15.

61. Goldschmidt, R.: Die sexuellen Zwischenstufen, Berlin, Julius Springer, 1931.

determine, but an effect of incompatibility of these traits themselves after they have developed. This is the case when an embryo of an Rh-positive blood group sensitizes the mother [62] whose blood does not contain Rh factor. As a rule, the embryo develops undisturbed unless the mother has previously been sensitized by Rh-positive substance, usually in the course of an earlier pregnancy, and has developed antibodies. These, transmitted to the embryo through the placenta, produce deleterious effects, the best known of which is the so-called erythroblastosis fetalis. Details of the ill effects of this constellation on the embryo will be described later. Other blood group incompatibilities between mother and fetus account for a small percentage of cases of erythroblastosis.[63]

There is a controversial and possibly highly important genetic cause of localized developmental abnormalities, namely, somatic mutation. This means that during the development of the embryo a mutation arises in one cell and is transmitted only to the descendants of that cell, which are thus genetically different from all other cells of the body. Most striking are those cases in which this appears to have happened at the first division of the fertilized egg cell, and one half of the body differs from the other in some genetically determined character. Examples are gynandromorphism,[63] unilateral gigantism [65] and unilateral pigmentary anomalies.[66] In arthropods genetic mosaics of various kinds are well known.[67] Another field in which somatic mutations have been taken into consideration is that of cancer.[68] This will be referred to in part III of this review.

ENVIRONMENTAL CAUSES OF MALDEVELOPMENT

It is obvious that abnormal development can be induced by any number of physical or chemical agents which will damage but not kill

62. Levine, P.; Burnham, E.; Katzin, E. M., and Vogel, P.: Am. J. Obst. & Gynec. **42**:925, 1941. Levine, P.: J. Pediat. **23**:656, 1943. Davidsohn, I.: J. A. M. A. **127**:633, 1945.

63. Polayes, S. H.: Am. J. Dis. Child. **69**:99, 1945.

64. Allen, E.; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, Baltimore, Williams & Wilkins Company, 1939.

65. (a) Mohr.[1e] (b) Hollander, W. F.: Quart. Rev. Biol. **19**:285, 1944. (c) Warren, D. C.: J. Hered. **36**:227, 1945. (d) Wartenberg, R.: Arch. Neurol. & Psychiat. **54**:75, 1945. (e) Zondek, L. H.: Arch. Dis. Childhood **20**:35, 1945. (f) Rugel, S. J.: Am. J. Dis. Child. **71**:530, 1946.

66. Zlotnokoff, M.: J. Hered. **36**:163, 1945. Glass, B.: ibid. **36**:192, 1945.

67. Goldschmidt.[61] Mohr.[1e]

68. (a) Furth, J.; Boon, M. C., and Kaliss, N.: Cancer Research **4**:1, 1944. (b) Furth, J., in Luck, J. M.: Annual Review of Physiology, Stanford University, Calif., Annual Reviews, Inc., 1944, vol. 6, p. 25. (c) Strong, L. C.: Arch. Path. **39**:232, 1945; Yale J. Biol. & Med. **18**:359, 1946.

the embryo. No attempt will be made here to review the large amount of older work in which various agents were applied to embryos at random and without regard to the mechanism of action. Great caution must be exerted in the evaluation of such work, because unknown accidental factors may be more potent than those intended to act. An example of this is the extensive work of Ferret,[69] who found that opening the shell of the hen's egg or manipulating the albumin of the egg has a profound influence on the embryo. Innumerable reports of experimental work with chick embryos have appeared since, and the necessary manipulations of the shell, of the albumin or even of the embryo itself have not produced the severe changes described by Ferret. On the other hand, workers in this field are often faced with the occurrence of numerous severe malformations in their material without an apparent cause. Shaking the eggs while they are being shipped to the laboratory has often been indicated and is certainly not without effect if it reaches a certain intensity. A few other causative agents have tentatively been identified—for example, fumes of a laboratory [70] or jarring.[71]

Mechanical Agents.—Displacement or destruction of parts of the embryo may be produced mechanically in accidental injuries or in surgical experiments. Comparable destructions are produced when parts of embryos are destroyed by chemical action or radiation, in contrast to true chemical or actinic action on development in which the affected tissue survives and shows the effect of the agent. Thus, electrolysis and roentgen rays have been used extensively in experimental embryology and teratology in order to eliminate certain tissues. The procedure is less hazardous than mechanical excision, and its effect on further development is essentially the same. Many of the common severe malformations, such as cyclopia or that of the sirenomelus, have been reproduced in chick embryos by localized roentgen ray destruction.[8c]

There are other kinds of mechanisms interfering with development in which the action is not so obvious. Numerous reports on invertebrates and lower vertebrates centrifuged early in their development will not be reviewed. A recent analysis of the literature and original work [72] relating to frogs revealed that at the early blastopore stage certrifugation is most effective in the production of malformations, and the optimum speed is about 2,500 revolutions per minute. Higher speeds cause early death. The malformations are varied and include, among

69. Ferret, P. E.: Arch. d'anat. micr. **7**:1, 1904.

70. Stockard, C. R.: Anat. Rec. **8**:33, 1914.

71. Stiles, K. A., and Watterson, R. L.: Anat. Rec. **70**:7, 1937.

72. Torrey, T. W., and Breneman, W. R.: Proc. Indiana Acad. Sc. **50**:213, 1941.

determine, but an effect of incompatibility of these traits themselves after they have developed. This is the case when an embryo of an Rh-positive blood group sensitizes the mother [62] whose blood does not contain Rh factor. As a rule, the embryo develops undisturbed unless the mother has previously been sensitized by Rh-positive substance, usually in the course of an earlier pregnancy, and has developed antibodies. These, transmitted to the embryo through the placenta, produce deleterious effects, the best known of which is the so-called erythroblastosis fetalis. Details of the ill effects of this constellation on the embryo will be described later. Other blood group incompatibilities between mother and fetus account for a small percentage of cases of erythroblastosis.[63]

There is a controversial and possibly highly important genetic cause of localized developmental abnormalities, namely, somatic mutation. This means that during the development of the embryo a mutation arises in one cell and is transmitted only to the descendants of that cell, which are thus genetically different from all other cells of the body. Most striking are those cases in which this appears to have happened at the first division of the fertilized egg cell, and one half of the body differs from the other in some genetically determined character. Examples are gynandromorphism,[63] unilateral gigantism[65] and unilateral pigmentary anomalies.[66] In arthropods genetic mosaics of various kinds are well known.[67] Another field in which somatic mutations have been taken into consideration is that of cancer.[68] This will be referred to in part III of this review.

ENVIRONMENTAL CAUSES OF MALDEVELOPMENT

It is obvious that abnormal development can be induced by any number of physical or chemical agents which will damage but not kill

62. Levine, P.; Burnham, E.; Katzin, E. M., and Vogel, P.: Am. J. Obst. & Gynec. **42**:925, 1941. Levine, P.: J. Pediat. **23**:656, 1943. Davidsohn, I.: J. A. M. A. **127**:633, 1945.

63. Polayes, S. H.: Am. J. Dis. Child. **69**:99, 1945.

64. Allen, E.; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, Baltimore, Williams & Wilkins Company, 1939.

65. (a) Mohr.[1e] (b) Hollander, W. F.: Quart. Rev. Biol. **19**:285, 1944. (c) Warren, D. C.: J. Hered. **36**:227, 1945. (d) Wartenberg, R.: Arch. Neurol. & Psychiat. **54**:75, 1945. (e) Zondek, L. H.: Arch. Dis. Childhood **20**:35, 1945. (f) Rugel, S. J.: Am. J. Dis. Child. **71**:530, 1946.

66. Zlotnokoff, M.: J. Hered. **36**:163, 1945. Glass, B.: ibid. **36**:192, 1945.

67. Goldschmidt.[61] Mohr.[1e]

68. (a) Furth, J.; Boon, M. C., and Kaliss, N.: Cancer Research **4**:1, 1944. (b) Furth, J., in Luck, J. M.: Annual Review of Physiology, Stanford University, Calif., Annual Reviews, Inc., 1944, vol. 6, p. 25. (c) Strong, L. C.: Arch. Path. **39**:232, 1945; Yale J. Biol. & Med. **18**:359, 1946.

the embryo. No attempt will be made here to review the large amount of older work in which various agents were applied to embryos at random and without regard to the mechanism of action. Great caution must be exerted in the evaluation of such work, because unknown accidental factors may be more potent than those intended to act. An example of this is the extensive work of Ferret,[69] who found that opening the shell of the hen's egg or manipulating the albumin of the egg has a profound influence on the embryo. Innumerable reports of experimental work with chick embryos have appeared since, and the necessary manipulations of the shell, of the albumin or even of the embryo itself have not produced the severe changes described by Ferret. On the other hand, workers in this field are often faced with the occurrence of numerous severe malformations in their material without an apparent cause. Shaking the eggs while they are being shipped to the laboratory has often been indicated and is certainly not without effect if it reaches a certain intensity. A few other causative agents have tentatively been identified—for example, fumes of a laboratory [70] or jarring.[71]

Mechanical Agents.—Displacement or destruction of parts of the embryo may be produced mechanically in accidental injuries or in surgical experiments. Comparable destructions are produced when parts of embryos are destroyed by chemical action or radiation, in contrast to true chemical or actinic action on development in which the affected tissue survives and shows the effect of the agent. Thus, electrolysis and roentgen rays have been used extensively in experimental embryology and teratology in order to eliminate certain tissues. The procedure is less hazardous than mechanical excision, and its effect on further development is essentially the same. Many of the common severe malformations, such as cyclopia or that of the sirenomelus, have been reproduced in chick embryos by localized roentgen ray destruction.[8c]

There are other kinds of mechanisms interfering with development in which the action is not so obvious. Numerous reports on invertebrates and lower vertebrates centrifuged early in their development will not be reviewed. A recent analysis of the literature and original work [72] relating to frogs revealed that at the early blastopore stage certrifugation is most effective in the production of malformations, and the optimum speed is about 2,500 revolutions per minute. Higher speeds cause early death. The malformations are varied and include, among

69. Ferret, P. E.: Arch. d'anat. micr. **7**:1, 1904.

70. Stockard, C. R.: Anat. Rec. **8**:33, 1914.

71. Stiles, K. A., and Watterson, R. L.: Anat. Rec. **70**:7, 1937.

72. Torrey, T. W., and Breneman, W. R.: Proc. Indiana Acad. Sc. **50**:213, 1941.

others, cyclopia and other defects of the head region, duplication of the caudal portion of the body and persistent open blastopore. The authors believe that dislocation of a portion of the organization center causes the defects of the head and the duplication of caudal parts.

An investigation of the effect of jarring on chick embryos [71] was precipitated by an unexplained increase in the occurrence of certain severe malformations in a laboratory in which other activities were also carried out. These malformations, including platyneuria (a peculiar form of nonclosure of the neural plate) and absence of yolk sac circulation, causing death, were reproduced by making a weight strike the table with the incubator many times in rapid succession during the early hours of incubation. In another series of experiments mechanical shaking of hen's eggs prior to incubation increased the incidence of all types of malformations, notably of "accidental" rumplessness.[73] In this case a combination of the mechanical factor and a genetic one was revealed, as the incidence of rumplessness was increased particularly in the offspring of those hens which were known to produce occasional rumpless chickens even without shaking.

In man and other mammals intrauterine development reduces the occurrence of mechanically caused malformations to a minimum. In the early phases of teratology, mechanical explanations were favored for almost all malformations and a narrow amnion or amniotic bands and adhesions were commonly indicated as the cause. Today investigators are more critical, and the incidence of these factors is found to be low when one relies on positive criteria, such as the demonstrable presence of amniotic bands and not just uncharacteristic furrows and irregular defects.[74] Moreover, some of the intrauterine "amputations" are known to be inherited in almost identical form,[75] which disproves an amniotic band causation. However, the occasional occurrence of malformations due to narrowing of the amniotic cavity (oligohydramnios) or to constriction of amniotic bands has been demonstrated beyond doubt.[74] That amniotic adhesions may be not accidental but sequelæ of defective conditions of the embryonic tissues, has been suggested by Streeter[76] and Seitz.[77] The histologic changes in the stumps of seemingly amputated legs of an infant have been described in detail by Taylor Gorostiaga and Lede.[78] The effect of constriction of em-

73. Landauer, W., and Baumann, L.: J. Exper. Zool. **93**:51, 1943.

74. Gruber, G. B., in Schwalbe, E., and Gruber, G. B.: Die Morphologie des Missbildungen des Menschen und der Tiere, Jena, Gustav Fischer, 1937, vol. 1, pt. 3, p. 278.

75. Koehler, O.: Ztschr. f. menschl. Vererb.- u. Konstitutionslehre **19**:670, 1936.

76. Streeter, G. L.: Contrib. Embryol. **22**:1, 1930.

77. Seitz, L.: Monatschr. f. Geburtsh. u. Gynäk. **94**:236, 1933.

78. Taylor Gorostiaga, D., and Lede, R. E.: Prensa méd. argent. **31**:363, 1944.

bryonic extremities, with subsequent rapid autolysis, has been investigated experimentally in mammals.[79]

Gross mechanical injury rarely leads to malformations in man and other mammals. If the injurious force penetrates the protecting envelopes, abortion or, in some mammals, resorption is the most probable outcome. In the recorded cases in which human embryo obviously survived an injury, the brain as the most vulnerable part is the most commonly affected. Pertinent examples are (1) apparently hemorrhagic destruction of both hemispheres following an accident during pregnancy [80] and (2) atypical presence of encephaloceles not in the embryonic line of closure of the primordium of the brain, with embolism and growth of brain tissue in the lungs.[81]

Mechanical conditions unfavorable for normal development may prevail in twins. Omphalocephaly [82] and ourentery [83] (anomalies in which the cranial and the caudal end of the body, respectively, grow into the yolk sac) of twins are probably caused by pressure of one twin on the other, in contrast to similar malformations of single embryos, which are probably not caused by external pressure.[84] The development of an acardiac twin may have a hydromechanic cause if one of twins, by virtue of his stronger circulation, takes over the function of propelling blood through the common placenta and the other twin. The latter will then suffer complete degeneration of large parts of his body. This course of events is hypothetic, and it is quite probable that at least in part of the cases the acardius is primarily maldeveloped or has abnormal vascular relations to his co-twin and the placenta which secondarily lead to the changes of circulation just described.[83]

Radiations.—An embryo may be deformed by radiation in two ways. One, in which a defect is caused by destruction of the irradiated tissue, has been mentioned in the preceding section. The other possibility is the survival of damaged tissue. While complete elimination of some tissue can usually not be ruled out, the following reports deal, in all probability, mainly with effects of the latter type. Hinrichs and Gentner [85] produced twins and double monsters in fish eggs with ultraviolet radiation, and determined as the most effective period the time just before the onset of cleavage. Many other malformations were also found, and various organs appeared to be affected to different degrees. The

79. Hellner, H.: Monatschr. f. Geburtsh. u. Gynäk. **95**:70, 1933.
80. Seitz, L.: Arch. f. Gynäk. **83**:701, 1907.
81. Gruenwald, P.: Am. J. Path. **17**:879, 1941. Potter, E. L., and Young, R. L.: Arch. Path. **34**:1009, 1942.
82. Gruenwald, P.: Ztschr. f. Anat. u. Entwcklngsgesch. **107**:782, 1937.
83. Gruenwald, P.: Anat. Rec. **83**:267, 1942.
84. Gruenwald, P.: J. Morphol. **69**:83, 1941.
85. Hinrichs, M. A., and Gentner, I. T.: Physiol. Zoöl. **4**:461, 1931.

circulatory and the central nervous system are more severely affected than other parts. Solberg[86] has reported on fish embryos that were exposed to roentgen rays. He distinguishes the following stages of the effect: (1) a latent period; (2) retardation through interference with mitosis; (3) disintegration of some tissues; (4) reorganization; (5) subsequent differentiation, depending on the changes previously produced. Uniform malformations can be obtained by properly controlled irradiation. Several workers have done similar work with amphibians.[87]

Von Hippel[88] produced cataracts in rabbit embryos by roentgen irradiation of the pregnant mother. Pagenstecher[89] obtained rosette formation in the retina by a similar procedure. Comparable effects have been observed in human embryos.[90] Experimentation with pregnant rats has yielded the following observations[91]: Hydrocephalus resulted most frequently from irradiation on the ninth day; ocular abnormalities, from that on the tenth day, and malformations on the jaws, from that on the eleventh day. In mice, irradiation on the seventh day resulted in resorption of embryos; that on the eighth day in meningocele; that on the ninth to fourteenth day, in kinked or short tail; that on the twelfth to fourteenth day, in hydrocephalus; that on the fourteenth to seventeenth day, in sterility, and that on the eighteenth and nineteenth day, in cataract.[92] This differential action may well be due to an effect of radiation proportionate to the growth rate at a given time and place,[93] although many other factors are probably involved as well. That roentgen rays have their predominant effect on cells in mitosis is well established.[14] A combination of irradiation and administration of ether[94] or increase of temperature[10b] has been reported to increase the effect in experimental animals.

Much has been reported on human embryos that received therapeutic doses of roentgen rays. In contrast to irradiation of the germ cells, which has not been proved to affect the offspring, irradiation of the embryo is responsible for a large number of cases of maldevelopment, which have been thoroughly investigated, notably microcephaly with

86. Solberg, A. N.: J. Exper. Zool. **78**:441, 1938.

87. Bardeen.[16b] Hertwig.[16c] Baldwin, W. M.: Anat. Rec. **17**:135, 1919; Am. J. Physiol. **52**:296, 1920; Anat. Rec. **22**:305, 1921. Curtis, W. C.; Cameron, J. A., and Mills, K. O.: Science **83**:354, 1936.

88. von Hippel, E.: Verhandl. d. deutsch. path. Gesellsch. **9**:174, 1905.

89. Pagenstecher, H. E.: Ber. ü. d. Versamml d. ophth. Gesellsch., 1916, p. 447.

90. (a) Lindenfeld, B.: Klin. Monatsbl. f. Augenh. **51**:443, 1913. (b) Goldstein, I., and Wexler, D.: Arch. Ophth. **5**:591, 1931; (c) **7**:434, 1932.

91. Job, T. T.; Leibold, G. L., Jr., and Fitzmaurice, H. A.: Am. J. Anat. **56**:97, 1935.

92. Kaven, A.: Ztschr. f. menschl. Vererb.- u. Konstitutionslehre **22**:238, 1938.

93. Woskressensky, N. M.: Arch. f. Entwcklngsmechn. d. Organ. **113**:447, 1928.

94. Haecker, V., and Lebedinsky, N.: München. med. Wchnschr. **61**:7, 1914.

mental deficiency and less frequently hydrocephalus, microphthalmia, malformations of the extremities and other parts.[95]   Retinal rosette formations comparable to those experimentally produced in the rabbit [89] have ·been studied in detail in embryos irradiated in order to terminate pregnancy.[90]   It has been found that well over 50 per cent of irradiated embryos (not counting those irradiated for intentional termination of pregnancy) suffer severe damage.[95b, c]   In view of this fact it has been suggested that pregnancy be interrupted if through unfortunate circumstances the embryo has received therapeutic doses of roentgen rays.[95a]   Diagnostic curettage of the uterus before irradiation of the organ during the generative period has also been advocated.[95c]   While in most of the reported cases the damage of the embryo was due to early irradiation, ill effects have been observed to follow treatment given during the latter half of intrauterine life.[96]   According to Bagg [97] and Goldstein and Murphy,[98] the effect of radium resembles that of roentgen rays.   It need not be elaborated in detail that these malformations are not hereditary, in contrast to those caused by mutation after irradiation of germ cells (see page 404).

Chemical Influences.—Countless investigations have dealt with chemical alterations of the embryo.   Only a few will be mentioned for their biologic or medical significance.   Much of the early work was done on the fish Fundulus heteroclitus, and striking modifications of development were obtained.   Werber [99] produced double monsters by means of acetone.   In addition, he [100] obtained, by administration of acetone or butyric acid, cyclopia and various malformations of the eyes, ears, olfactory pits, mouth, central nervous system, heart and vessels, fins, tail and body form.   There are also most interesting cases in which isolated eyes or lenses were found on the blastoderm far from the embryo itself.[101]   This led Werber [101b] to develop the concept of blastolysis, which means an action of chemicals resulting in destruction or in

95. (a) Murphy, D. P.; Shirlock, M. E., and Doll, E. A.: Am. J. Roentgenol. **48**:356, 1942. (b) Zappert, J.: Wien. klin. Wchnschr. **38**:669, 1925; Arch. f. Kinderh. **80**:34, 1926. (c) Murphy, D. P.: Am. J. Obst. & Gynec. **18**:179, 1929; (d) footnote 21. (e) Feldweg, P.: Strahlentherapie **26**:799, 1927. (f) Goldstein, L., and Murphy, D. P.: Surg., Gynec. & Obst. **50**:79, 1930. (g) Flaskamp, W., in Meyer, H.: Sonderbünde zur Strahlentherapie, Berlin, Urban & Schwarzenberg, 1930, vol. 12, p. 1. (h) Sternberg, H.: Chir. d. org. di movimento **24**:231, 1939. (i) Maxfield, F. N.: Am. J. Ment. Deficiency **45**:358, 1941. (j) Glass, S. J.: J. Clin. Endocrinol. **4**:47, 1944.

96. Goldstein, L., and Murphy, D. P.: Am. J. Roentgenol. **22**:322, 1929.

97. Bagg, H. J.: Am. J. Anat. **30**:133, 1922.

98. Goldstein, L., and Murphy, D. P.: Am. J. Obst. & Gynec. **18**:189, 1929.

99. Werber, E. I.: J. Exper. Zool. **24**:409, 1917.

100. Werber, E. I.: Anat. Rec. **9**:529, 1915; footnote 6 c.

101. Werber, E. I.: (a) J. Exper. Zool. **21**:347, 1916; (b) Anat. Rec. **10**:258, 1916.

splitting and dispersion of the germ. As this work was based on experimental use of acetone and butyric acid, which may be present in the human circulation under abnormal conditions, it was suggested [102] that human malformations may have the same cause. While this deduction cannot be subscribed to, for many obvious reasons, and the mechanism of Werber's blastolysis is not understood, the interesting facts remain and have been powerful stimuli for further work. Stockard used inorganic salts in experiments on the same species and found that magnesium salts produced a remarkable incidence of cyclopia. The most constant effect on various embryonic structures was obtained with lithium salts. These produced a retardation of development, malformations of the eyes, colorless (sic) blood and a slow heart rate.[103] McClendon [6d] examined the power of many inorganic and organic compounds to produce cyclopia, and along with the chemical properties of these agents he considered also their surface tension.

In regard to amphibians the work done with lithium salts overshadows all other experiments with chemicals in extent and importance. Lehmann [104] found the notochord absent and the myotomes (somites) fused across the midline in locations depending on the times at which the subjects were exposed to the salts. Similar results were obtained by Cohen,[105] and the original articles should be consulted for the somewhat different explanations given by the two workers. In the head region, not only faulty differentiation or arrangement of the primordia occurs, but severe defects up to cyclopia or complete anophthalmia with associated lesions of the brain. All these malformations are not specific for the action of lithium; they occur, though less regularly, after the use of other chemicals [106] and after an increase of temperature.[107] The action of trichlorbutyl-alcohol on lens formation in amphibians has also been investigated in great detail and with particular attention to the effect of various concentrations.[108]

An interesting and severe malformation in amphibians, which occasionally occurs spontaneously, has been produced with increased frequency by raising embryos in 0.35 per cent sodium chloride solution.[109] It is exogastrulation, an abnormality of the process of gastrulation in which the mesoderm, instead of moving into the interior at the blastopore, moves outward to form a separate mass. In extreme

102. Werber, E. I.: Anat. Rec. **9**:133, 1915; Bull. Johns Hopkins Hosp. **26**:226, 1915; footnote 6 c.

103. Stockard, C. R.: J. Exper. Zool. **3**:99, 1906; footnote 6 a.

104. Lehmann, F. E.: Arch. f. Entwcklngsmechn. d. Organ. **136**:112, 1937; **136**:106, 1938.

105. Cohen, A.: J. Exper. Zool. **79**:461, 1938.

106. Lehmann.[104] Cohen.[105]

107. Hoadley, L.: Growth **2**:25, 1938.

108. Lehmann, F. E.: Arch. f. Entwcklngsmechn. d. Organ. **131**:333, 1934.

109. Holtfreter, J.: Arch. f. Entwcklngsmechn. d. Organ. **129**:669, 1933.

cases it may constrict itself off from the ectoderm, and the embryo is then divided into two separate pieces. Just what the action of the salt is in provoking this process is not understood. Exogastrulation has also appeared occasionally after other experimental procedures.

In frog embryos 2,4-dinitrophenol produces a general retardation of development and any of the following malformations: persistent yolk plug, absence of external gills, papillary outgrowths of the epidermis and abnormalities of the eyes and the neural tube.[110]

There are many reports on the action of chemicals on the chick embryo. Various substances acting chemically or as foreign bodies were administered by Bauer [111] and Canat and Opie.[112]. The former reports hypoplasia of the mesenchyme and inhibition of the outgrowth of peripheral nerves, supposedly consecutive to the disturbance of the mesenchyme. Ectodermal and entodermal structures are normal or retarded, or show foci of necrosis. Canat and Opie examined the local inflammatory reaction to the injection of india ink or turpentine. In embryos of 3 to 5 days the most prominent reaction is cell proliferation. Phagocytosis also occurs early. Granulocytes begin to appear at the end of the first week. Shortly before birth, inflammation assumes the well known postnatal forms. That scarlet red induces epithelial proliferations and irregularities of the neural tube has been claimed,[113] but not confirmed in a later investigation.[114] It is not known whether or not this discrepancy was due to differences in the chemical nature of the dye or to its impurities. Alcohol in suitable concentrations produces tachycardia in 48 hour embryos, but no malformations beyond disturbances of the curvature of the body.[115] Colchicine produces not only the well known abnormalities of mitosis ("colchicine figures") but also malformations of the neural tube [116] or, according to other authors,[117] strophosomus (an extreme dorsal flexion of the spine). When colchicine is applied to certain circumscribed parts of the embryo, dwarfed limbs may be obtained, as well as reduced numbers of digits.[118] Landauer [119] observed in chick embryos, after injecting Ringer solution into the eggs, an increased prenatal and a decreased postnatal mortality. This is

110: Dawson, A. B.: J. Exper. Zool. **78**:101, 1938.

111. Bauer, K.: Virchows Arch. f. path. Anat. **294**:477, 1935.

112. Canat, E. H., and Opie, E. L.: Am. J. Path. **19**:371, 1943.

113. Waelsch, L.: Arch. f. Entwcklngsmechn. d. Organ. **38**:509, 1914. I had an opportunity to see serial sections of Waelsch's specimens and to confirm the presence of the malformations described in this article.

114. Burnier, J., and Sauser-Hall, P.: Compt. rend. Soc. de biol. **116**:927, 1934.

115. Petry, E., and Ferrier, A.: Compt. rend. Soc. de biol. **116**:928, 1934.

116. Paff, G. H.: Am. J. Anat. **64**:331, 1939.

117. Lallemand, S.: Compt. rend. Acad. d. sc. **207**:1446, 1938. Ancel, P., and Lallemand, S.: ibid. **210**:710, 1940. Gabriel, M. L.: J. Exper. Zool. **101**:339, 1946.

118. Gabriel, M. L.: J. Exper. Zool. **101**:339, 1946.

119. Landauer, W.: Poultry Sc. **8**:301, 1929.

apparently an instance in which mostly the weaker ones, which would succumb to other influences after birth, are killed as embryos. As was mentioned in a foregoing paragraph, a similar explanation has been given for the action of alcohol on the germ cells.[31]. Landauer [119] also found that lithium salts and, to a less extent, magnesium salts cause a great increase of mortality shortly before hatching, but no malformations were seen. In another series of experiments, rumplessness was produced by insulin and several other organic compounds.[120] Catizone and Gray [121] have reported three types of distortion of the head following administration of lead compounds. The published views of whole embryos suggest that the malformations are all platyneuria, which occurs frequently in the laboratory without intentional interference. Other workers [122] found general retardation of growth and relatively greater retardation of somite formation. The development of head and eyes is inhibited. *In both investigations the embryos were not sectioned.* Gray and Worthing [123] injected tetanus toxin and observed a profound influence on the central nervous system and the head of the early embryo. These malformations, too, are of those types which frequently occur without apparent cause.

Of great economic importance is a series of investigations originally designed to discover the cause of so-called alkali disease of farm animals in the Middle West.[124] It was found that the disease is a poisoning due to consumption of selenium-containing plants. In connection with the present subject, only the findings in chick embryos are of interest. Mature chickens are not much affected by selenium· in their diet, but embryos of selenium-poisoned hens show severe disturbances.[125] A high percentage of them fail to hatch and exhibit, among others, malformations of the brain, the eyes (including cyclopia), the beak and the extremities or an "edemic" (sic) condition. Similar malformations have been produced by injecting selenium compounds into eggs.[126] In the course of the same investigation arsenic, fluorine and lead compounds were also tested and failed to cause comparable disturbances. These substances caused a high embryonic death rate, also ectopia of the viscera.[126] Landauer [49] found that selenium-induced malformations share with certain others a predilection for one side of the body (eyes and wings of the left side; legs of the right side).

120. Landauer, W.: J. Exper. Zool. **98**:65, 1945. Landauer, W., and Lang, E. H.: ibid. **101**:41, 1946. Landauer, W., and Bliss, C. I.: ibid. **102**:1, 1946.

121. Catizone, O., and Gray, P.: J. Exper. Zool. **87**:71, 1941.

122. Hammett, F. S., and Wallace, V. L.: J. Exper. Med. **48**:659, 1928.

123. Gray, P., and Worthing, H.: J. Exper. Zool. **86**:423, 1941.

124. Moxon, A. L.: Bulletin 311, South Dakota Agricultural Experiment Station, 1937, p. 1.

125 Franke, K. W., and·Tully, W. C.: Poultry Sc. **14**:273, 1935.

126. Franke, K. W.; Moxon, A. L.; Poley, W. E., and Tully, W. C.: Anat. Rec **65**:15, 1936.

Few investigations have been undertaken to examine in mammals the influence on the embryo of substances injected into pregnant females. In recent years drugs have been tested in order to determine whether, if used during pregnancy, they might endanger the embryo. Sulfanilamide was tested in rats.[127] The concentration was equal in the maternal and the fetal blood and higher than that used in therapy. Prolonged administration increased the mortality before and after birth. The birth weight was diminished and postnatal growth retarded. Penicillin, on the other hand, had no detrimental effect on the embryo under the conditions of an independent investigation.[128] Thiourea was similarly examined.[129] The thyroid gland of the embryo showed the histologic changes characteristic of thiourea treatment. No malformations were recorded. Strontium compounds administered to pregnant rabbits replace calcium in fetal bones and produce a condition of "pseudorickets".[130] In rat embryos whose mothers receive a diet containing 25 per cent galactose cataracts develop. The changes resemble postnatal galactose cataract.[131] Alloxan has no direct effect on the rat embryo.[132] Hansmann and Perry [133] in a series of unselected cases in which there was no exposure to industrial hazards found lead in 62.5 per cent of human fetuses examined. In 25 per cent the amounts were considered dangerous. However, it is stated that the fetus is protected by the growing skeleton which binds the lead. The authors emphasize the possibility of abortion due to lead. No malformations were observed. Only one report has been found concerning the possibility that the fetus might be damaged by arsenicals used in antisyphilitic treatment during pregnancy.[134]

In the preceding pages the effects of foreign substances on embryonic development are reviewed. It is to be expected that striking effects would be produced in the embryo by those organic substances which even in the more stabile mature organism control structural changes, namely, hormones. It is unfortunate that Gley's [135] subdivision of these

127. Speert, H.: Bull. Johns Hopkins Hosp. **66**:139, 1940.

128. Greene, H. J., and Hobby, G. L.: Proc. Soc. Exper. Biol. & Med. **57**:282, 1944.

129. Goldsmith, E. D.; Gordon, A. S., and Charipper, H. A.: Am. J. Obst. & Gynec. **49**:197, 1945.

130. Lehnerdt, F.: Beitr. z. path. Anat. u. z. allg. Path. **46**:468, 1909.

131. Bannon, S. I.; Higginbottom, R. M.; McConnell, J. M., and Kaen, H. W.: Arch. Ophth. **33**:224, 1945.

132. Friedgood, C. E., and Miller, A. A.: Proc. Soc. Exper. Biol. & Med. **59**:61, 1945.

133. Hansmann, G. H., and Perry, M. C.: Arch. Path. **30**:226, 1940.

134. Arnold, W.: Virchows Arch. f. path. Anat. **311**:1, 1944.

135. Gley, E.: The Internal Secretions: Their Physiology and Application to Pathology, New York, Paul B. Hoeber, 1917.

active substances into hormones proper and harmozones, proposed in the early days of endocrinology, has not been generally accepted. According to Gley's definition, substances of the former group control functional activity, whereas harmozones control morphogenetic processes. Disturbances in the balance of harmozones should affect the embryo profoundly. The relationship of these agents to embryonic organizers has been discussed by Needham.[4]

The older literature on the endocrine glands of the fetus and their interrelation with one another, with other parts of the embryo and with agents in its environment has been reviewed by Thomas.[136] One important concept dating from that early period is that of synkainogenesis,[137] a term which designates all endocrine interactions between mother and embryo. Well known examples of this are the stimulation of the mammary glands of the newborn by maternal lactogenic hormone and the hyperplasia of the interstitial cells of the testes of the embryo, which is well marked in man [138] and reaches tremendous proportions, also those of the ovaries, in horse embryos.[139] Endocrine abnormalities of the mother may influence the embryo. Peculiarities of the children of diabetic mothers have recently been studied in great detail. They include increased birth weight, enlargement of the heart, extramedullary erythropoiesis, hyperplasia of the pancreatic islands and changes in other endocrine organs.[140] It was thought that the metabolic disturbance and the insulin deficiency of the mother were directly responsible for these changes. However, it has now been found that infants of mothers in whom diabetes develops only some time after termination of pregnancy have similar changes.[141] This has not been explained as yet. The fetal and neonatal mortality is increased during a five year period preceding the onset of maternal diabetes.[142]

Hereditary dwarfism of mice, apparent at birth, is probably mediated by an abnormality of the pituitary gland.[143] Mongoloid deficiency has been tentatively correlated with maternal and fetal pituitary dysfunc-

136. Thomas, E.: Innere Sekretion in der ersten Lebenszeit (vor und nach der Geburt), Jena, Gustav Fischer, 1926.

137. Kohn, A.: Arch. f. Entwcklngsmechn. d. Organ. 39:112, 1914.

138. Mita, G.: Beitr. z. path. Anat. u. z. allg. Path. 58:554, 1914. Neumann, H. O.: Ztschr. f. Geburtsh. u. Gynäk. 90:100, 1930. Diaca, C.: Virchows Arch. f. path. Anat. 304:171, 1939.

139. Kohn, A.: Ztschr. f. Anat. u. Entwcklngsgesch. 79:366, 1926. Petten, J. L.: ibid. 99:338, 1933.

140. (a) Potter, E. L.; Seckel, H. P. G., and Stryker, W. A.: Arch. Path. 31:467, 1941. (b) Miller, H. C.; Johnson, R. D., and Durlacher, S. H.: J. Pediat. 24:603, 1944.

141. Miller, H. C.: Am. J. M. Sc. 209:447, 1945.

142. Miller, H. C.: J. Pediat. 29:455, 1946.

143. Greene, H. S. N.: J. Exper. Med. 71:839, 1940.

tion.[144]   On the other hand, human subjects with pituitary dwarfism usually produce normal offspring.[145]   Fetal thyroid or iodine deficiency produces congenital cretinism.[146]   The mental development of persons with congenital hypothyroidism, particularly that of endemic cretins, often fails to respond adequately to administration of thyroid.   It has therefore been suggested that damage of the brain may not be merely a consequence of thyroid' deficiency but an associated lesion.[147]

A possible correlation of abnormalities of endocrine organs of the embryo is the repeatedly investigated severe atrophy of the adrenal cortex of the anencephalic monster.[148]   It has not been definitely established whether or not this condition is caused by an abnormality of the hypophysis in the anencephalic monster, as was claimed by Kohn.[148b]   I have seen a similar atrophy of the adrenal cortex in a stillborn infant with hydrocephalus and abnormalities at the base of the brain. No pituitary gland could be found (case unpublished).

By far the largest amount of work in embryonic endocrinology has been done with estrogenic and androgenic substances.   It is of great interest because many of the experiments have resulted in abnormalities related to intersexuality.   Work done up to 1939 has been reviewed by a group of outstanding experts.[64]   Since then, experimental work has been done with the opossum,[149] the mouse,[150] the rat[151] and the monkey.[152]   In all classes of vertebrates the genital organs and other sexually different characteristics may be transformed into forms more or less resembling those of the opposite sex by proper application of estrogen or androgen to embryos.   A strong tendency of the genetic sex to assert itself and condition the effect of estrogen or androgen was noted in some of these experiments but until recently hormones were generally

144. (a) Benda, C. E.; Dayton, N. A., and Prouty, R. A.: Am. J. Psychiat. 99:822, 1943. (b) Beidleman, B.: Am. J. Ment. Deficiency 50:35, 1945.

145. Speck, G.: Am. J. Obst. & Gynec. 51:217, 1946.

146. Benda, C. E.: Mongolism and Cretinism, New York, Grune & Stratton, Inc., 1946.

147. Bruch, H., and McCune, D. J.: Am. J. Dis. Child. 67:205, 1944.

148. (a) Meyer, R.: Virchows Arch. f. path. Anat. 210:158, 1912. Landau, M.: Verhandl. d. deutsch. path. Gesellsch. 16:301, 1913.   (b) Kohn, A.: Arch. f. Entwcklngsmechn. d. Organ. 102:113, 1924. Angevine, D. M.: Arch. Path. 26:507, 1938.

149. (a) Moore, C. R.: Physiol. Zoöl. 14:1, 1941. (b) Burns, R. K., Jr.: Biol. Symposia 9:125, 1942.

150. Raynaud, E.: Compt. rend. Acad. d. sc. 205:1453, 1937; Compt. rend. Soc. de biol. 127:503, 1938. Turner, C. D.: J. Morphol. 65:353, 1939.

151. (a) Hamilton, J. B., and Wolfe, J. M.: Anat. Rec. 70:433, 1938. (b) Greene, R. R.; Burrill, M. W., and Ivy, A. C.: Am. J. Anat. 65:415, 1939; 67:305, 1940. (c) Greene, R. R.: Biol. Symposia 9:105, 1942.

152. van Wagenen, C., and Hamilton, J. B., in Essays in Biology in Honor of Herbert M. Evans, Berkeley, University of California Press, 1943, p. 581.

believed to play a dominating part in the morphogenesis of the genital organs (except the gonads themselves) under normal as well as under abnormal conditions. From experiments carried out during the last few years it has been concluded by some leading authorities that the gonads of the embryo do not produce hormones until after the genital tract is well established in male or female form. Even the gonadotropic hormone of the pituitary gland fails to stimulate the gonads in these stages to secrete hormones.[153] This is in accord with pathologic findings in human cases of congenital absence or severest hypoplasia of the gonads [154] in which the genital tract is definitely differentiated in the direction of one sex. In later periods sexual differentiation will, of course, suffer in gonadless individuals. The power of the genetic sex to assert itself, even in postnatal periods, is well illustrated in experiments in which male chick embryos were completely feminized by administration of estrogen. They had histologically normal ovaries, and yet were reconverted to the male sex after birth if estrogen treatment was not continued.[155] Thus the leading role of the genetic sex in determining not only the sexual form of the gonads but also that of the entire organism has come to be increasingly appreciated.

It is of great medical importance to realize the tenacity of the genetic sex. It explains the fundamental difference between genetic and hormonal intersexuality. In the former the sexual development is abnormal by the standards of the usual male or female organization but normal for the individual itself in that it conforms with that individual's genetic pattern. Hormonal intersexes, on the other hand, have a sex which is genetically normal by the usual standards but which is secondarily changed by hormones. These individuals will by themselves tend to revert to their genetic sex if the hormonal imbalance is eliminated. In addition, there is probably a condition of genetically determined hormonal intersexuality which may, if not properly analyzed, be confused with genetic intersexuality. It is produced by the action of genetically determined hormonal imbalance in individuals with a normal genetic sex. This occurs in genetically caused or conditioned growths with heterosexual hormonal activity. Familial intersexuality associated with hyperplasia or tumor of the adrenal cortex [156] probably belongs to this group.

---

153. Moore, C. R.: Am. Naturalist **78**: 97, 1944; J. Clin. Endocrinol. **4**:135, 1944.

154. Kermauner, F., in Halban, J., and Seitz, L.: Biologie und Pathologie des Weibes, Berlin, Urban & Schwarzenberg, 1924, vol. 3, p. 281. Rössle, R., and Wallart, J.: Beitr. z. path. Anat. u. z. allg. Path. **84**:401, 1930. Pich, G.: ibid. **98**:218, 1937. Erskine, C. A., and Rannie, I.: Arch. Path. **42**:381, 1946.

155. Wolff, E.: Arch d'anat., d'histol. et d'embryol. **23**:1, 1936. Dantchakoff, V.: Compt. rend. Acad. d. sc. **202**:1112, 1936.

156. Werthemann, A.: Schweiz. med. Wchnschr. **71**:1335, 1941.

While the domain of the sex hormones proper may not be as great in normal embryonic development as was formerly believed, it is quite possible that chemical correlations, perhaps more of the organizer type, are active in the early development of the genital organs. Here must be mentioned Witschi's [157] hypothetic substances which govern female development of the germ cells in the cortex, and male development in the medulla, of the gonad. Of undetermined normal significance is a hormone-like substance which produces a highly perplexing type of intersexuality in certain mammals: The freemartins among cattle are individuals with abnormal sex development, which are always twins of normal males. It was discovered simultaneously and independently by Keller and Tandler [158] and Lillie [159] that the abnormal twin is a genetic female modified by a substance transmitted from the male twin through anastomoses in their chorionic circulations. A considerable number of detailed investigations have since substantiated this theory and elucidated the development of the freemartin. Similar intersexual twins also occur among pigs and goats but not among heterosexual twins with vascular anastomoses in the cat, the peludo and the marmoset (see Witschi's review [160]). Moore [153] points out that conditions strictly comparable with those observed in the freemartin have not as yet been produced in experiments, and he concludes that the problem of the freemartin is just as mysterious now as ever. Marsman [161] compared freemartins with genetic intersexes and geldings as to morphologic aspects and excretion of hormones. His conclusion is that the freemartin is an individual deprived early of its sources of sex hormones and more thoroughly than the gelding.

It remains to mention the effect of certain tumors of the cortex of the adrenal gland on the genital tract. In a small proportion of cases of corticoadrenal tumors for which no specific histologic characteristics have yet been found, as well as in some instances of hyperplasia of the adrenal cortex, the female subjects may be masculinized at any period of prenatal or postnatal life (interrenalism [162]). This effect disappears promptly on removal of the excessive adrenal tissue unless sex transformation in early embryonic life has produced irreversible changes.[163] A much smaller number of cases in which men were

157. Witschi, E.: Biol. Rev. **9**:460, 1934.

158. Keller, K., and Tandler, J.: Wien. tierärztl. Wchnschr. **3**:513, 1916.

159. Lillie, F. R.: Science **43**:611, 1916.

160. Witschi, E., in Allen, Danforth and Doisy,[64] p. 145.

161. Marsman, W. S.: Acta neerland. morphol. **1**:115, 1937.

162. Mathias, E.: Zentralbl. f. Gynäk. **50**:2489, 1926. Berner, O., in Hirsch, M.: Handbuch der inneren Sekretion, Leipzig, Curt Kabitzsch, 1927, vol. 25, p. 1143.

163. McKenna, C. M.; Kiefer, J. H., and Bronstein, I. P.: Tr. Am. A. Genito-Urin. Surgeons **35**:41, 1943.

feminized by similar growths have been reported.[164]. Familial occurrence has already been mentioned.[158] Various theories have been proposed concerning the androgenic activity (neglecting the few cases in which feminization occurred) of one or another of the normal constituents of the adrenal cortex and he relationship of the just mentioned tumors to these. None of the theories is supported by convincing evidence, and they will therefore not be reviewed. Somewhat less problematic is the influence on sex of various tumors of organs which normally produce sex hormones, as the testis, the ovary and the placenta. This subject is adequately treated in many texts of pathology or of gynecology. A case of masculinization of a female fetus by an ovarian tumor of the mother, probably an arrhenoblastoma, is on record.[165]

Deficiencies.—If any of essential substances is lacking during embryonic life, this may produce severe developmental disturbances. Some of these disturbances have been investigated in chick embryos, partly because their occurrence in commercial hatching is of considerable economic importance. Micromelia was observed in chick embryos, caused by a deficient diet of the hen, by Byerly and co-workers,[166] and its morphologic aspects were studied by Landauer.[167] Lyons and Insko [168] and Caskey and Norris [169] have described the same manifestations in manganese deficiency and they, and later Landauer,[170] have assumed that the first-mentioned deficiency was also one of manganese. Gallup and Norris [171] found that manganese deficiency produces increased mortality of chick embryos. The embryos are fully developed but unable to hatch. The authors call this congenital debility in analogy to findings in the rat (see page 428). The influence of vitamin deficiencies has been examined in chick embryos. Low vitamin A content of the egg results in poor hatchability.[172] Riboflavin deficiency causes degeneration of the mesonephros, edema, anemia and abnormal down.[173] Another report mentions lethal changes in

164. Schiller, W.: Internat. Clin. **3**:87, 1940.

165. Brentnall, C. P.: J. Obst. & Gynaec. Brit. Emp. **52**:235, 1945.

166. Byerly, T. C.; Titus, H. W.; Ellis, N. R., and Landauer, W.: Proc. Soc. Exper. Biol. & Med. **32**:1542, 1935.

167. Landauer, W.: Anat. Rec. **64**: 267, 1936.

168. Lyons, M., and Insko, W. M., Jr.: Bulletin 371, Kentucky Agricultural Experiment Station, 1937, p. 61.

169. Caskey, C. D., and Norris, L. C.: Proc. Soc. Exper. Biol. & Med. **44**:332, 1940.

170. Landauer, W.: Sigma Xi Quart. **28**:171, 1940.

171. Gallup, W. D., and Norris, L. C.: Poultry Sc. **18**:83, 1939.

172. Bearse, G. E., and Miller, M. W.: Poultry Sc. **16**:39, 1937.

173. Lepovsky, S.; Taylor, L. W.; Jukes, T. H., and Almquist, H. L.: Hilgardia **11**:559, 1938.

the extraembryonic vessels ("lethal ring") and chondrodystrophy and other defects in those embryos which survive.[174] A third group [175] claims that the first changes to appear are those in the nervous system, including degeneration of myelin sheaths. These can be prevented by injecting riboflavin into the eggs. Vitamin D deficiency seems to occur in chick embryos as indicated by the high mortality in eggs of hens kept in closed rooms, which can be reduced by giving the hens cod liver oil.[176] Vitamin E deficiency causes retardation of development, and death through degeneration of vessels, rupture of the atrium of the heart or of large vessels, and impairment of growth of the allantois, which is essential for respiration.[177] However, the pertinence of these observations has been questioned,[178] because it is not certain that they are the effect of vitamin E deficiency alone. Lack of biotin is followed by increased embryonic mortality at certain stages, chondrodystrophy and syndactyly.[179]

Considerable experimental work has been reported on the effect of vitamin deficiencies on mammalian embryos. Many references have been gathered by Mason.[180] The same author has reported in detail on the results of vitamin A deficiency in pregnant rats [181]: Early death of many embryos, followed by resorption, results from inflammatory changes of the placenta. The surviving embryos are retarded in growth, gestation is often unduly prolonged, and many newborn young die soon. In the pig severe defects of the hindlegs follow maternal deficiency of a fat-soluble factor,[182] which Needham [4] states is vitamin A. In another investigation definite lack of this vitamin caused microphthalmia in every young one throughout several experiments, but no malformations of the legs.[183] In the rat, ocular malformations of a different kind are observed in association with maternal vitamin A deficiency.[184] A series of reports describes the development of cleft palate and malformations of the extremities of the embryos of rats

174. Romanoff, A. L.: Anat. Rec. (supp.) **78**:78, 1940.

175. Engel, R. W.; Phillips, P. H., and Halpin, J. G.: Poultry Sc. **19**:135, 1940.

176. Insko, W. M., and Lyons, M.: Bulletin 363, Kentucky Agricultural Experiment Station, 1936, p. 64.

177. Adamstone, F. B.: J. Morphol. **52**:47, 1931.

178. Mason, K. E.: Yale J. Biol. & Med. **14**:605, 1942.

179. Cravens, W. H.; McGibbon, W. H., and Sebesta, E. E.: Anat. Rec. **90**:55, 1944.

180. Mason, K. E., in Allen, Danforth and Doisy,[64] p. 1149.

181. Mason, K. E.: Am. J. Anat. **57**:303, 1935.

182. daZilva, S. S.; Golding, J.; Drummond, J. C., and Coward, K. H.: Biochem. J. **15**:427, 1921.

183. Hale, F.: Texas State J. Med. **33**:228, 1937.

184. Warkany, J., and Schraffenberger, E.: Proc. Soc. Exper. Biol. & Med. **57**:49, 1944; Arch. Ophth. **35**:150, 1946.

maintained on a certain deficient diet.[185]   These disturbances can be prevented with dried pig liver.   The same malformations result from riboflavin deficiency.[186]   Similar, but not identical, defects were produced by feeding the mother a rachitogenic diet.[187]   Avitaminosis C (deficiency of ascorbic acid) has been produced in guinea pig embryos.[188]   Research on the effect of vitamin E deficiency started with the work of Evans, Burr and Althausen [189] and Urner.[190]   Early abnormal development of the mesenchyme and general retardation were observed, as well as reduction of the entodermal villi of the placenta and impairment of the blood islands.   The result was death of the fetus.   Mason [191] described in hypovitaminotic embryos which survived to the sixteenth day a hemorrhagic state, with much blood *accumulated in the vessels of the skin, and cerebral hemorrhages.* He attributes the lack of cells in the blood-forming tissues to this escape of blood to the periphery.

Manganese deficiency produces in rats a state of "congenital debility" which renders them unable to live through the period of birth, even though they appear normal in utero shortly before term.[192]

Vitamin deficiencies of human embryos and newborn infants have repeatedly been reported.[193]   In most cases neither the type of deficiency nor that of the structural changes has been clearly defined.   The conditions found were called fetal rickets or osteomalacia, but no typical entities have as yet been demonstrated.   The possible significance of vitamin deficiencies is indicated by the suggestion of Balfour and Talpade [194] that the high infant mortality of India may be caused by a deficiency, possibly of the vitamin B complex.   Lack of iron in tuber-

---

185. Warkany, J., and Nelson, R. C.: Anat. Rec. **79**:83, 1941; Arch. Path. **34**:375, 1942. Warkany, J.; Nelson, R. C., and Schraffenberger, E.: Am. J. Dis. Child. **64**:860, 1942.

186. Warkany, J., and Schraffenberger, E.: Proc. Soc. Exper. Biol. & Med. **54**:92, 1943.

187. Warkany, J.: Am. J. Dis. Child. **66**:511, 1943.

188. (*a*) Ingier, A.: J. Exper. Med. **21**:525, 1915.   (*b*) Reyher, P.; Walkhoff, E., and Walkhoff, O.: München. med. Wchnschr. **75**:2087, 1928.

189. Evans, H. M.; Burr, G. O., and Althausen, T. L.: The Antisterility Vitamine Fat Soluble E, Memoirs of the University of California, Berkeley, University of California Press, 1927, vol. 8, p. 1.

190. Urner, J. A.: Anat. Rec. **50**:175, 1931.

191. Mason, K. E., in Essays in Biology in Honor of Herbert M. Evans, Berkeley, University of California Press, 1943, p. 401.

192. Daniels, A. L., and Everson, J. G.: J. Nutrition **9**:191, 1935.

193. (*a*) Reyher, Walkhoff and Walkhoff.[188b] (*b*) Maxwell, J. P.; Hu, C. H., and Turnbull, H. M.: J. Path. & Bact. **35**:419, 1932.   (*c*) Akamatu, K.: Okayama-Igakkai-Zasshi **52**:979, 1940.

194. Balfour, M. I.: Indian M. Gaz. **65**:630, 1930.   Balfour, M. I., and Talpade. S. K.: ibid. **67**:601, 1932.

culous mothers and their fetuses has been suggested as the cause of anemia and decreased resistance of the newborn.[195] ·Iodine deficiency produces in mammals congenital goiter and hairlessness [196] and in man goiter and deaf-mutism.[197] Stott [198] attributes similar findings to an excess of calcium in the water rather than to iodine deficiency, or to a disproportion of calcium and iodine. Closely related conditions were mentioned in a foregoing section under thyroid deficiency.

Detrimental effects of an unspecified nutritional deficiency of the mother on the fetus are suggested by Burke, Beal, Kirkwood and Stuart,[199] who state that in 216 cases studied, "every stillborn infant, every infant who died within a few days of birth, with the exception of one, the majority of infants with marked congenital defects, all premature and all 'functionally immature' infants were born to mothers whose diet during pregnancy was very inadequate." In view of the large number of other known causes of the conditions in question, one may not agree with this statement to its full extent; yet, it is perhaps not quite true that the fetus always obtains the best possible "diet" even at the expense of the mother, as has often been stated.

Changes in Concentration of Oxygen and Carbon Dioxide.—If the concentrations of oxygen and carbon dioxide in the environment of incubating chicken eggs are decreased or increased, respectively, beyond certain levels this interferes with normal development of the embryo. According to Romanoff,[200] concentrations of carbon dioxide up to 0.4 per cent are compatible with normal development and may even stimulate growth. According to the same investigator, concentrations of carbon dioxide higher than 1 per cent with a corresponding reduction of oxygen produce slow growth, various malformations and early death. If the exposure to air of abnormal composition is temporary, concentrations up to 22 per cent carbon dioxide with 16 per cent oxygen are compatible with continued growth. Another author [201] obtained a high percentage of embryos with platyneuria (a peculiar form of nonclosure of the neural tube) by preventing the exchange of gas through the egg shell with a coat of wax. In other experiments embryos exposed to an atmosphere containing 22 to 37 per cent

195. Albers, H.: Arch. f. Gynäk. **172**:173, 1941.

196. Smith, G. E.: J. Biol. Chem. **29**:215, 1917. Welch, K.: Bulletin 119, University of Montana Agricultural Experiment Station, 1917, p. 81. Hart, E. B., and Steenbock, H.: J. Biol. Chem. **33**:313, 1918.

197. Brain, W. R.: Quart. J. Med. **20**:303, 1927. Murray, M. M., and Wilson, D. C.: Nature, London **155**:79, 1945.

198. Stott, H.: Indian J. M. Research **20**:147, 1932.

199. Burke, B. S.; Beal, V. A.; Kirkwood, S. B., and Stuart, H. C.: Am. J. Obst. & Gynec. **46**:38, 1943.

200. Romanoff, A. L.: J. Morphol. **50**:517, 1930.

201. Gallera, J.: Folia morphol. **6**:203, 1936.

carbon dioxide presented platyneuria in all instances. The critical stage was found to last from the appearance of the primitive streak until the beginning formation of the notochord and neural plate. Byerly[202] described in somewhat older embryos as suffocation effect large blood vessels dilated into sinuses of excessive dimensions and death of the tissues in the deeper layers of the germ. I have seen the same abnormalities as "spontaneous" malformations in eggs which had not intentionally or knowingly been subjected to anoxia, which suggests anoxia as a natural cause of malformations. Anoxia due to failure of extraembryonic blood circulation to develop properly is thought to be responsible for some of the malformations and their peculiar lateral distribution in prothanic homozygous Creeper chick embryos.[46] The left eye, which is commonly more affected than the right, is turned away from the surface of the blastoderm by the normal rotation of the head and thus receives less oxygen by diffusion while the development of circulation lags. Transplantation experiments have shown that the primordia of the right and left eyes of these embryos are potentially equal.[203] Experimental obstruction of the extraembryonic circulation in genetically normal embryos leads to similar malformations with severer manifestations on the left side of the head, whereas an increase of the oxygen tension attenuates the defects of the left eye in prothanic Creeper embryos.[46] A subsequent examination of normal chick embryos incubated under favorable conditions showed that the left eye lags temporarily in the majority of them.[18] This asymmetry is present only during a short period beginning when the head turns to the side, and ending when an effective circulation is established. This is well in accord with the assumption of transitory anoxia of the left eye. A great preponderance of malformations of the left eye exists not only in Creeper embryos but also among sporadic[204] and selenium-induced malformations.[49] It may be assumed that temporary anoxia is a contributing factor which decreases the resistance of the left eye to other teratogenic agents.[18]

It is obvious that anoxia cannot be of great importance as a teratogenic factor in mammals. One can imagine that disturbances of oxygen supply occur before nidation in the uterus, or before the beginning of embryonic circulation, but this has not been observed. In later stages certain poisons may interfere with the metabolism of oxygen. A pertinent case of carbon monoxide poisoning of a pregnant woman has been described.[205] in which the mother recovered, while

202. Byerly, T. C.: Anat. Rec. **32**:249, 1926.

203. Gayer, K.: J. Exper. Zool. **89**:103, 1942.

204. (*a*) Gruenwald.[18] (*b*) Landauer, W.: Anat. Rec. **86**:365, 1943.

205. Maresch, R.: Wien. med. Wchnschr. **79**:454, 1929.

the child, which was born thirteen days after the poisoning and lived nine days, showed severest softening of the thalami and the corpora striata. In guinea pigs, anoxia preceding delivery produces severe cerebral damage.[206] In man, temporary anoxia of the older fetus stimulates respiratory movements, and may endanger the fetus by causing aspiration of amniotic fluid containing sebaceous material, cornified cells or meconium.[207] Damage of the brain similar to that observed in the aforementioned animal experiments may cause mental deficiency.[208]

Abnormal Temperature.—This environmental factor has no known teratologic importance in man and other mammals. Several authors have examined the influence of an abnormal incubation temperature in chick embryos and found various structural defects.[209] In the same species the expression of some hereditary malformations has been modified by a lowered temperature, as was mentioned.[210]

Petersen [211] has reported studies of the influence of various climatic factors on the incidence of malformations in man.

Infection and Inflammation.—The question whether maldevelopment may be caused by infection and subsequent inflammation leads to the frontiers of what is commonly considered to be maldevelopment as opposed to disease. That no strict borderline exists between these two divisions of the field of pathology, has been emphasized in the introduction.

The possible role of inflammation as a cause of abnormal development has been much discussed with respect to malformations of the heart. In the past it was believed that many of these malformations were due to embryonic endocarditis, but this explanation is no longer accepted for the great majority of cases. Gross [212] goes so far as to

206. (*a*) Windle, W. F.; Becker, R. F., and Weil, A.: J. Neuropath. & Exper. Neurol. **3**:224, 1944. (*b*) Windle, W. F., in Harvey Lectures, Lancaster, Pa., Science Press, 1945, vol. 40, p. 236.

207. (*a*) Farber, S., and Sweet, L. K.: Am. J. Dis. Child. **42**:1372, 1931. (*b*) Helwig, F. C.: Am. J. Obst. & Gynec. **26**:849, 1933. (*c*) Snyder, F. F., and Rosenfeld, M.: ibid. **36**:363, 1938. (*d*) Warwick, M.: New York State J. Med. **37**:2075, 1937.

208. Schreiber, F.: J. A. M. A. **111**:1263, 1938. Benda, C. E.: Am. J. Psychiat. **97**:1135, 1941. Clifford, S. H.: J. Pediat. **18**:567, 1941. Lamm, S. S.: Am. J. Ment. Deficiency **48**:131, 1943. Raymond, C. S.: ibid. **49**:8, 1944. Benda, C. E.: Medicine **24**:71, 1945.

209. Danforth, C. H.: Proc. Soc. Exper. Biol. & Med. **30**:143, 1932. Romanoff, A. L.: Poultry Sc. **15**:311, 1936. Smith, L. E. W.: Arch. Path. **28**:422, 1939.

210. Sturkie.[52] Landauer.[51]

211. Petersen, W. F.: Am. J. Obst. & Gynec. **28**:443, 1934; Am. J. Orthodontics **27**:179, 1941.

212. Gross, P.: Arch. Path. **31**:163, 1941.

conclude from his own case and a review of the literature that there is no proved instance of fetal endocarditis, and others agree with him.[213] On the other hand, several authors [214] have described cases of acute endocarditis of undoubtedly prenatal origin. I have seen severe endocarditis of the tricuspid valve in an infant less than 1 day old.[215] These cases do not bear directly on the question of the inflammatory origin of cardiac malformations, as they concern late stages of intrauterine life. However, if one considers the relatively extremely small number of early embryos which are adequately examined (i.e. in serial sections), one must realize that failure to find acute endocarditis allows no conclusions. Fetal myocarditis has been found repeatedly.[216] Farber and Hubbard [216b] point out that of two groups into which congenital abnormalities of the heart may conveniently be divided, the one comprising gross departures from the normal developmental plan is not likely to be due to endocarditis. The other group includes hearts with normal septums and the typical relations of the large vessels and shows stenosis or atresia of valves, and these abnormalities are thought to be the effects of inflammation. I have found indications of a past inflammation, similar to those described by Farber and Hubbard, in the heart of a stillborn infant with atresia of the pulmonary ostium and in a newborn infant with stenosis of the mitral and aortic valves (cases unpublished).

The investigation of malformations of the heart and other organs occurring frequently in the infants of mothers who contracted rubella during the early months of pregnancy [217] may well contribute important information as soon as embryonic stages are examined. If the disease occurs during the first or second month of pregnancy, the inci-

213. Weintraub, T., and Himmelfarb, A. J.:   Bull. Johns Hopkins Hosp. **72:** 299, 1943.

214. Plaut, A., and Sharnoff, G.: Arch. Path. **20:**582, 1935. Püschel, E.: Arch. f. Kinderh. **114:**1, 1938. Plaut, A.: Am. J. Path. **15:**649, 1939. MacGregor, R. R., and McKendry, R.: Canad. M. A. J. **50:**433, 1944.

215. Dr. A. J. Gitlitz gave me the opportunity to see this specimen.

216. (*a*) Stoloff, E. G.: Am. J. Dis. Child. **36:**1204, 1928.   (*b*) Farber, S., and Hubbard, J.: Am. J. M. Sc. **186:**705, 1933.

217. Swan, C.; Tostevin, A. L.; Moore, B.; Mayo, H., and Black, G. H. B.: M. J. Australia **2:**201, 1943. Swan, C.; Tostevin, A. L.; Mayo, A., and Black, G. H. B.: ibid. **1:**409, 1944. Erickson, C. A.: J. Pediat. **25:**281, 1944. Reese, A. B.: Am. J. Ophth. **27:**483, 1944. Gregg, N. M.: M. J. Australia **1:**313, 1945. Welch, L. S. V.: ibid. **1:**574, 1945. Long, J. C., and Danielson, R. W.: Arch. Ophth. **34:**24, 1945. Greenthal, R. M.: Arch. Pediat. **62:**53, 1945. Albaugh, C. H.: J. A. M. A. **129:**719, 1945. Carruthers, D. G.: M. J. Australia **1:**315, 1945. Prendergast, J. J.: Arch. Ophth. **35:**39, 1946. Guerry, D.: Am. J. Ophth. **29:**190, 1946. Goar, E. L. and Potts, C. R.: ibid. **29:**566, 1946. Swan, C., and Tostevin, A. L.: M. J. Australia **1:**645, 1946. Aycock, W. L., and Ingalls, T. H.: Am. J. M. Sc. **212:**366, 1946.

dence of malformations is nearly 100 per cent. Most commonly affected are the eyes (cataract, microphthalmia), the brain (mental retardation, microcephaly) and the heart. Only a few cases have as yet been studied at autopsy.[218] In contrast to most other authors, Fox and Bortin [219] conclude from their investigation of epidemics of rubella in Milwaukee that many pregnancies are unaffected. Conte, McCammon and Christie [220] sent 120 questionnaires to the mothers of infants with malformations of those types which may be caused by rubella. Of 61 mothers who answered, 5 gave a history of having had rubella during pregnancy. In view of the large number of other possible causes of the malformations in question, this is a significantly high incidence.

The developmental changes in fetal syphilis are too well known to be discussed here. Numerous reports deal with less common agents infecting the embryo—with virus,[221] bacteria [222] and protozoa.[223] Particularly tubercle bacilli have received a great deal of attention.[224] Localization of infectious organisms in the placenta is important in the mechanism of spreading of some of the diseases due to them, par-

218. Swan, C.: J. Path. & Bact. **56**:289, 1944; Tr. Ophth. Soc. Australia **4**: 132, 1944.

219. Fox, M. J., and Bortin, M. M.: J. A. M. A. **130**:568, 1946.

220. Conte, W. R.; McCammon, C. S., and Christie, A.: Am. J. Dis. Child. **70**:301, 1945.

221. (*a*) Reuss, A., in Halban, J., and Seitz, L.: Biologie und Pathologie des Weibes, Berlin, Urban & Schwarzenberg, 1927, vol. 8, p. 2. (*b*) Lynch, F. W.: Arch. Dermat. & Syph. **26**:997, 1932. (*c*) Brody, H.: New York State J. Med. **41**:1256, 1941. (*d*) Woolpert, O. C.: Am. J. Path. **12**:141, 1936. Gallagher, F. W., and Woolpert, O. C.: J. Exper. Med. **72**:99, 1940. Goodpasture, E. W.: Science **95**:391, 1942. Goodpasture, E. W., and Anderson, K.: Am. J. Path. **18**:563, 1942.

222. Reuss.[221a] Wohlwill, F., and Bock, H. E.: Arch. f. Gynäk. **135**:211, 1929; Beitr. z. path. Anat. u. z. allg. Path. **85**:469, 1930. Burn, C. G.: Am. J. Path. **12**:341, 1936. Kelley, R. W.: Arch. Path. **28**:248, 1939. Diddle, A. W., and Stephens, R. L.: Am. J. Obst. & Gynec. **38**:300, 1939. Roback, H. N., and Kahler, H. F.: J. Nerv. & Ment. Dis. **94**:669, 1941. Price, S., and Chang, T.: Arch. Path. **41**:450, 1946.

223. (*a*) das Gupta, B. M.: Indian M. Gaz. **74**:397, 1939. (*b*) Paige, B. H.; Cowen, D., and Wolf, A.: Am. J. Dis. Child. **63**:474, 1942. (*c*) Koch, F.; Wolf, A.; Cowen, D., and Paige, B. H.: Arch. Ophth. **29**:1, 1943. (*d*) Zuelzer, W. W.: Arch. Path. **38**:1, 1944. (*e*) Pratt-Thomas, H. R., and Cannon, W. M.: Am. J. Path. **22**:779, 1946.

224. Whitman, R. C., and Greene, L. W.: Arch. Int. Med. **29**:261, 1922. Schmorl, G., in Engel, S., and Pirquet, C.: Handbuch der Kindertuberkulose, Leipzig, George Thieme, 1930, vol. 1, p. 137. Siegel, M.: Am. Rev. Tuberc. **29**:297, 1934. Siegel, M., and Singer, B.: Am. J. Dis. Child. **50**:636, 1935. Conrad, C. A.: South. M. J. **32**:169, 1939. Schaefer, G.: Quart. Bull., Sea View Hosp. **4**:457, 1939. Schwartz, R., and Gerhart, O.: Compt. rend. Soc. de biol. **129**:1095, 1939. Schenck zu Schweinsberg, H. G.: Monatschr. f. Kinderh. **88**:145, 1941. Loewenstein, E.: Am. Rev. Tuberc. **51**:225, 1945.

ticularly tuberculosis. As far as the investigators know, developmental disorders are but rarely caused by these infections. However, the example of rubella shows how unsuspected correlations between embryonic infection and malformation may become apparent and their medical importance appreciated once they are brought to attention. Cellular inflammatory reaction to infection in embryonic tissues has been examined in experimental and clinical material.[225]

COMMENT

The knowledge of the causes of malformations reviewed in the preceding pages is based largely on experiments. Part of it, being based on experiments with lower vertebrates, does not apply directly to man and other mammals with their excellent protection of the embryo. It has been reviewed not only in order to illustrate general principles of teratology but also because the resulting malformations are often comparable in more or less detail with mammalian ones. The development of the malformation in an experimental animal shows how a similar condition in man may have arisen. This comparison cannot offer more than a suggestion, since it is known that different mechanisms may lead to similar final results. Incidental findings of a few early stages in man may then support or refute the comparison. Moreover, the time of action may be more important than the exact nature of a teratogenic agent, as will presently be discussed. Thus the action of agents which in themselves do not occur in man and other mammals may be duplicated by others which exert the same influence (e.g., inhibition of development) at a comparable time and place in the embryo.

It has repeatedly been postulated that the action of detrimental agents on development depends to a considerable degree on intrinsic circumstances in the embryo. As an example, the previously mentioned temporary anoxia of the left side of the head of the chick embryo may be recalled. It not only produces a transitory lag in the normal development of the left eye but is presumably the cause of the marked preponderance of sporadic malformations of the left eye of the chick embryos.[18] More often a rapid rate of growth and development has been regarded as a predisposing factor,[1a] and it is on this basis that investigators have explained how nonspecific detrimental factors acting on the entire embryo during limited periods may produce well defined

225. Wohlwill, F., and Bock, H. E.: Virchows Arch. f. path. Anat. 291:864, 1933. Lillie, R. D.: Pub. Health Rep. 50:1498, 1935. Goldsworthy, N. E., and Moppett, W.: J. Path. & Bact. 41:529, 1935. Goodpasture, E. W., and Anderson, K.: Am. J. Path. 13:149, 1937. Buddingh, G. J., and Polk, A. D.: J. Exper. Med. 70:485 and 499, 1939. Canat, E. H., and Opie, E. L.: Am. J. Path. 19:385, 1943.

malformations only in certain organs.   Child [226] and his followers have developed the concept of gradients of vital activities in developing organisms and have shown how these gradients influence normal and abnormal development.   Thus Hyman [227] has discussed the influence of high rates of growth at given points on the effects of injurious agents.   The defect produced is more severe in a part whose rate of growth is higher than those of others exposed to the same agent.   Up to a certain limit, this may be compensated by a great ability of the rapidly growing part to make restitution of itself.   Beyond that limit, the permanent damage will be greater in a part with a high growth rate than elsewhere.   Thus a pattern of sensitivity exists in the developing organism at a given stage which may be responsible for identical reactions to widely differing teratogenic agents, genetic and environmental. Not only can different abnormal genes produce the same malformation,[54] but exact phenocopies [228] of hereditary abnormalities may be produced by agents affecting the tissues of the individual itself and not its genetic constitution.[229]   The concept has evolved that at least part of the teratogenic genes affect the embryo in a nonspecific manner at a constant stage of embryonic development.   This agrees well with recent work suggesting that much if not all of gene action affects metabolic processes.[12]   It has been pointed out that phenocopies have been produced only of those hereditary traits which are assumed to be caused by a change in the relative rates of developmental processes (which are of greater importance in the production of malformations).   On the other hand, no phenocopies are on record of traits which interfere with definite steps of chemical synthesis.[230]   Perhaps this is so because the former act but once, whereas the latter act throughout life and cannot very well be imitated by extrinsic agents.   The assumption of · an action of genes on metabolism is supported by several previously mentioned observations.   Lowering the incubation temperature and thus the rate of growth of the embryo at a given time can reduce its reaction to gene action and diminish the expression of certain hereditary traits in the chick.[210]   The cumulative effect of the hereditary Creeper factor and selenium poisoning in the chick embryo has been explained by the assumption that both interfere in a similar manner with metabolic activity.[49]

226. Child, C. M.: Am. Naturalist **58**:237, 1924; Patterns and Problems of Development, Chicago, University of Chicago Press, 1941.

227. Hyman, L. H.: Biol. Bull. **40**:32, 1921.

228. A phenocopy is an abnormality closely resembling a certain hereditary trait, but produced by an influence on the individual itself, and not hereditary.

229. (*a*) Cairns.[46] (*b*) Kaven, A.: Ztschr. f. menschl. Vererb.- u. Konstitutionslehre **22**:247, 1938; footnote 92.

230. Goldschmidt, R. B.: J. Exper. Zool. **100**:193, 1945.

It is apparent that malformations are the effect of a variety of intrinsic and extrinsic, synergistic or antagonistic agents. Many of these act in a nonspecific manner and may be replaced by others with similar action without changing the final result.

In the preceding pages one may find several instances of discrepancies in the results obtained by different workers with the same agent. This may be due to differences in the technic of the experiment or in the genetic constitution of the animals used. It is obvious from what has just been said that slight variations of the strength of the agent and particularly of the timing of its action may change the results fundamentally. The interaction of genetic and environmental factors in the production of malformations has been referred to on several occasions, and this may explain variations in the results of experiments with different strains of animals.

There is a method of approach to malformations and their causative agents which has not yet been mentioned in this review, namely, statistics. It is quite possible that in the future many questions of the action of hereditary or environmental influences on the developing organism will be answered by statistical evaluation of large groups of cases. Particularly concerning human maldevelopment, results might be obtained which would otherwise not be available, as experimental methods cannot be used. A detailed study of a large number of human cases correlated with information on various aspects of environment and other factors has been made by Murphy.[11] The only consistent result so far obtained in several independent statistical investigations is a striking difference between white persons and Negroes in the over-all incidence of malformations; the incidence is higher in white persons.[231]

---

231. Murphy.[11] Potter, E. L.: J. A. M. A. **115**:996, 1940. Gruenwald, P.: Illinois M. J. **79**:55, 1941.

(To Be Continued)

Notes and News

State Toxicologic Service.—A toxicologic service has been organized in the department of pharmacology and toxicology of the University of Illinois College of Medicine, of Chicago, under the direction of C. C. Pfeiffer. This service will be available to the offices of state's attorney and coroner in all the counties of Illinois. Similar services are used in Michigan, Indiana and Wisconsin.

Careers in Academic Medicine.—An opportunity to start a career in academic medicine is offered by the John and Mary R. Markle Foundation, 14 Wall Street, New York 5, to young scientists with the necessary training to hold regular faculty appointment and to conduct research. The new program of postfellowship grants will be conducted in cooperation with accredited medical schools of the United States and Canada. Grants of $25,000, to be paid to the cooperating school at the rate of $5,000 annually toward the support of each successful candidate or his research or both, will be available, beginning with the academic year 1948-1949. If the plan proves successful, the foundation will appropriate a total of $1,250,000 payable to the schools by 1953. Candidates will be recommended by medical schools, and recommendations will be limited to young men and women who show a particularly strong interest in research and in teaching in any of the clinical or preclinical sciences or in the sciences basic to medicine. They will have had training in some special field or combination of fields to qualify them to receive a regular faculty appointment and to conduct original research. The final choice of "Scholars in Medical Science" will be made, on the basis of the schools' recommendations, by regional committees appointed by the foundation. About fifty will receive appointments during the five year period. The school will determine salary and academic rank, encourage research by setting reasonable limits on non-research activities, provide laboratory facilities and, if necessary, make a financial contribution toward support of the scholar's work. The program is the result of a survey of medical research and education recently made by the foundation, which shows that while there are scholarships and other forms of financial aid for the student in the course of his scientific training, and while there are funds available to the scientist once his name is made, there are few sources of help at the beginning of the career of the man who chooses academic medicine. Persons interested in being considered as candidates are referred to deans of accredited medical schools for further information.

Maternal Infections and Congenital Malformations. — The American Academy of Pediatrics and the National Society for the Prevention of Blindness have organized a nationwide survey to collect data which may establish a relationship between infection in the expectant mother and the occurrence of congenital defects in the offspring. Measles, chickenpox, mumps and influenza are examples of maternal infections. Physicians knowing of such cases are asked to register them with Dr. Herbert C. Miller, professor of pediatrics, University of Kansas School of Medicine, Kansas City, Kan.

The World Federation of Pathologists.—The European Association of Clinical Pathologists has reconstituted itself as a world federation of societies devoted to clinical pathology. The federation aims (1) to develop clinical pathology,

which is the application of pathology and allied sciences to medicine, (2) to have regard to the scientific and professional status of those engaged in its practice, and (3) to foster international amity. A committee will call a meeting of delegates from as many interested associations as possible next November in Paris, during the meeting of the French Société de Biologie Clinique, to formulate a constitution and elect officers. The federation invites societies devoted to clinical pathology to communicate with the secretaries—Dr. W. H. McMemnemy, pathologic department, Royal Infirmary, Worcester, England, or Dr. J. Ungar, Glaxo Laboratories, Ltd., Greenford, Middlesex, England. Medical practitioners or university graduates practicing clinical pathology who are members of the constituent bodies will thereby become members of the federation. Membership will be available also to those who are eligible but do not belong to a constituent body. The federation proposes to hold a conference at least once every three years at the same time and place as the summer meeting of one of its constituent bodies. It hopes that the first meeting will take place in the summer of 1948.

CORRECTION

An error was made in numbering the legends for figures 1 and 2 in the article by Major Welland A. Hause and Captain Gunnard J. Antell entitled "Arteriosclerosis in Infancy," which appeared in the July issue (ARCH. PATH. **44**:82, 1947). Under figure 1 the legend designated *B* should be *A;* that designated *A* should be under figure 2, on page 85. The legend under figure 2 is the legend for 1 *B*.

The magnification of figure 2 is × 235, instead of × 70.

Books Received

A Handbook for the Diagnosis of Cancer of the Uterus by the Use of Vaginal Smears. By Olive Gates, M.D., pathologist, Massachusetts State Tumor Diagnosis Service; assistant pathologist, Pondville Hospital (Massachusetts Department of Public Health), and Shields Warren, M.D., assistant professor of pathology, Harvard Medical School; pathologist, New England Deaconess and New England Baptist hospitals; reserve consultant in pathology to the United States Navy Bureau of Medicine and Surgery, Captain (M.C.) U.S.N.R. With a Foreword by George N. Papanicolaou, M.D., Ph.D., associate professor, Department of Anatomy, and research associate, Department of Obstetrics and Gynecology, Cornell University Medical College and New York Hospital. Pp. 182, illustrated. Cambridge, Mass.: Harvard University Press, 1947.

This book should find a ready audience among physicians who, in increasing numbers, are becoming interested in the vaginal smear used as an adjunct of the existing means of diagnosing cancer of the uterine corpus and cervix. It begins with an attempt to evaluate the usefulness of the procedure and subsequently gives the methods of preparing the smear, a brief description of the normal histologic pattern of the female reproductive organs and a discussion of the criteria warranting a diagnosis of cancer. It concludes with 50 excellent plates, each made up of from 2 to 6 photomicrographs showing the representative types of cells found in the vaginal smear.

The value of the vaginal smear as a means of making a diagnosis of cancer earlier than would otherwise be possible has not yet been established, and, as the authors point out, a conclusion can be reached only after large numbers of careful observations have been made and adequately evaluated. Two tables presented by the authors are especially interesting. One concerns 6,265 cases in which vaginal smears had been made. Among the 475 cases in which carcinoma of the uterus was found, no symptoms were present in 8, and smears were positive in 8, although the first biopsies gave negative results. The other table includes 5,583 cases, 490 of which were proved to be cases of cancer. In this group 120 erroneous diagnoses were made on vaginal smears; in 63 of these a false positive diagnosis was made, and in 57 the diagnosis was not made even though cancer was eventually proved to be present.

The authors point out the difficulties attendant on the attempt to make a diagnosis of cancer from one or a few cells and refer to the time-tested belief that the general structure of tissue, as well as the individual cell pattern, must be taken into account in arriving at a conclusion. They stress the time-consuming nature of the examinations and state that a minimum of twenty minutes should be allowed for each smear unless an obvious carcinoma is present and that at times an adequate examination requires an hour. They believe that an untrained person of average aptitude who is studying for two hours daily under good supervision will require four months to a year to attain proficiency in diagnosis.

The descriptions of the various types of normal and abnormal cells found in vaginal smears are detailed and should be of much help to the inexperienced observer. The photomicrographs are well reproduced and will be found of value. It is always difficult, however, to obtain an adequate idea of cell structure from photographs, and, excellent as these are, the average observer will find many cells in actual smears that he will be unable to identify by use of the plates alone.

It is refreshing to find a book devoted to a relatively new subject which so dispassionately presents and analyzes the material. It is carefully planned, well written and comprehensive in scope. It could be read with profit by all physicians interested in the diagnosis of cancer of the uterus.

CANCER: DIAGNOSIS, TREATMENT AND PROGNOSIS. By Lauren V. Ackerman, M.D., pathologist to the Ellis Fischel State Cancer Hospital and assistant professor of pathology at Washington University School of Medicine, St. Louis, and Juan A. del Regato, M.D., radiotherapeutist to the Ellis Fischel State Cancer Hospital and formerly assistant to the Radium Institute of the University of Paris. Pp. 1,115 with 745 test illustrations and 42 color reproductions. Price $20. St. Louis: C. V. Mosby Company, 1947.

In spite of the progress made in surgical and radiation therapy during the last twenty-five years, the over-all picture allows little improvement of results of cancer therapy to be recognized. Only a statistically negligible minority of patients with curable cancers have the benefit of adequate therapy without avoidable delay. One of the main reasons for this situation is the lack of coordination of teaching of the different phases of cancer—pathology, biology, diagnosis, therapy—in medical schools, so that "even the better than average interne frequently lacks adequate understanding of malignant disease" (National Advisory Cancer Council, 1946).

The book by Ackerman and Regato is eminently fitted to help fill this gap. This book is to this reviewer's knowledge the most comprehensive, most reliable and, for practical purposes, best balanced presentation available for the use of the undergraduate student, the general practitioner and the specialist.

The smaller general part of the book contains chapters entitled "Pathology of Cancer," "Surgery of Cancer" and "Radiotherapy of Cancer." Each one of these gives a synopsis of the clinically pertinent points which in practice frequently are not appreciated in their true importance. These chapters are preceded by a short introduction, in which some statistical facts are discussed, and a chapter on cancer research contributed by Michael B. Shimkin, of the National Cancer Institute, which gives an instructive summary of the trends and accomplishments noted in the research field to date.

While the general chapters contain many most useful practical suggestions, the selection of which is obviously derived from the daily experience of the authors, their composition is somewhat haphazard—at times too specialized for the general reader, yet not detailed enough for the specialist, as for instance some physical and technical discussions of radiation therapy. The chapter entitled "Pathology of Cancer" is probably the one best integrated in the general context of the book. Remarks about the technic of obtaining a specimen for biopsy and the necessity of submitting sufficient data for its evaluation are much to the point. The responsibility of the pathologist and the limitations of his services are briefly but precisely discussed. The conservative attitude of the authors with reference to the clinical importance of tumor grading is most satisfying.

The second part of the book is devoted to the cancers of different organs in detail. The length of the chapters varies greatly and is not commensurate with the incidence of the tumor under consideration. It was obviously determined by the practical necessity of distributing greater knowledge of some less generally known aspects of the more curable forms of cancer, and the more important recent developments have received priority on space. Likewise is there a marked difference in the quality and the originality of different chapters, naturally depending on difference in the personal experience and particular interest of the authors.

The outstanding chapter, of almost 300 pages, "Cancer of the Respiratory System and Upper Digestive Tract," is based on the unusually great personal experience of the authors in this field. In it they discuss in detail the forms of cancer of the sinuses, the oral cavity, the lip, the pharynx and the larynx. This field, as daily experience teaches, is still quite confusing to many physicians' minds as far as precise diagnosis and understanding of the relative indications of surgical and radiation therapy and the possible accomplishments are concerned. This chapter represents, probably, the most comprehensive and authentic presentation of this particular field available, and if it could be published as an independent monograph for the use of the respective specialists, that would be highly desirable. A careful study of the detailed discussion of the types of tumors encountered from the point of gross and microscopic pathology and exact location as well as from the point of accessibility to surgical treatment or irradiation should greatly help to clarify the situation, and this in turn undoubtedly would lead to an increased rate of cure of these tumors, the curability of which is high if they are recognized early and treated properly.

Two other excellent chapters are those on cancer of the skin and cancer of the female genital organs. In the discussion of treatment of cutaneous cancers the emphasis on the care with which treatment—surgical and radiologic—is to be adjusted to the individual requirements, dependent on location and extent of the disease, with abandonment of any routine procedure applied to all cases, is particularly to the point and will help to prevent some of the failures which may make a curable cancer incurable.

In the discussion of cancers of the female genital organs, the important portion on carcinoma of the cervix is precise and well balanced. In spite of the by now fairly well standardized situation, much can be learned from careful study of this chapter. Particularly, the detailed description of the clinical staging (supported by excellent illustrations of the types of lesions encountered in the different stages) will help to clarify a still quite prevalent confusion which was partly caused by the change of the League of Nations classification in 1937 and thus facilitate an intelligent appreciation of results of treatment. The practical suggestions concerning certain details of the pelvic examination of the cancerous patient as contrasted with the routine gynecologic examination (the examination with both the right and the left hand, the technic of the examination of the rectovaginal septum, examination with the speculum preceding detailed palpation) are only a few of the many points which demonstrate the meticulous attention of the authors to details that are important in practice. Likewise does such attention to details of the individualization of treatment lead the authors to refute the myth of radioresistant cancers of the cervix by the statement, "No carcinoma arising from the cervix is radioresistant. Even adenocarcinomas, which for many years were judged less amenable to radiotherapy, have long been recognized as radiosensitive and radiocurable." The now again prevalent and frequently exaggerated controversy of radiotherapy against surgery is reduced to its true proportions by the observation that only 10 per cent of the total number of patients would be suitable for surgical treatment.

The other common forms of cancers of the female genital organs are adequately discussed, but one misses a discussion of endometriosis, which, particularly in its stromal variety, may be important for differential diagnosis. Most of the previously diagnosed "sarcomas" of the uterus probably belong in this group and a more general knowledge of this curable disease would be desirable.

In contrast to these outstanding and original chapters, one finds some chapters dealing with the more commonly known varieties of cancer in a more conventional

way. Still the information given is based on a careful evaluation of the particular experiences either of the authors themselves or of other workers. Considerable, on the whole undisputed, information is thus presented under the headings: "Tumors of the Thyroid Gland"; "Tumors of the Mediastinum"; "Cancer of the Digestive Tract"; "Cancer of the Genito-Urinary Tract"; "Cancer of the Male Genital Organs"; "Tumors of the Suprarenal Gland"; "Cancer of the Mammary Gland"; "Malignant Tumors of Bone"; "Sarcomas of the Soft Tissues."

Some short chapters on cancer of the eye, Hodgkin's disease and leukemia conclude the text.

To each chapter is appended a bibliography of the sources cited in the text.

Each one of the special chapters discusses anatomy, incidence and etiology, pathology, clinical evolution, diagnosis, treatment and prognosis. While such rigid organization could easily have led to an academic enumeration of facts, it is the educationally outstanding and truly unique feature of this book that the authors succeeded in intelligently coordinating anatomy, pathology and clinic. Accordingly, the emphasis varies considerably in different chapters, depending on the practical importance of the different phases. One of the most important features is the detailed discussion of the lymphatic channels of each organ, the precise knowledge of which is so important for rational management. Excellent sketches facilitate understanding of this subject.

Every phase of the discussion is profusely illustrated. The most instructive illustrations are the excellent sketches prepared for the elucidation of the text. The photographic illustrations are in general adequate for the demonstration of the point in question, though by no means technically brilliant. While most of the illustrations add to understanding, some—like those of far advanced, hopeless lesions—represent, rather, museum pieces without didactic value. These as well as all the color plates should have been omitted. The color plates are poorly executed, and none of them adds to the educational value. Their elimination may reduce the price of the book and thus increase its distribution and usefulness.

The enormous amount of material is presented in a detached and unbiased way. Everywhere one can recognize the critical evaluation of the authors' own matured experiences, with proper integration and critical evaluation of the opinions and experiences of other workers. Although the book is written by a pathologist and a radiologist, there is nowhere undue emphasis on the possibilities and accomplishments of radiation therapy. In a way it is regrettable that no surgeon has cooperated —not that this would have improved the presentation or would have changed the statements as to the relative place of irradiation and surgical procedure, which are most conservative—but it might have given more weight to the conclusions in the minds of those who still do not fully appreciate the place of irradiation for certain forms of cancer. While the book is not primarily meant for the cancer specialist— it contains little technical detail—the wealth of the authors' experiences and their intelligent and critical evaluations make fascinating reading for the experienced specialist in the field, who as a gourmet will enjoy many of the intellectual delicacies. As is to be expected with a book of this scope, there will be minor disagreements respecting some of the authors' opinions. As a whole, however, the book represents one consistent unit which reflects the prolonged close cooperation of the authors and their profound understanding of the problems involved. This work in itself again demonstrates the value of such close cooperation in the field of cancer therapy. If it is used as much as it deserves, it will represent one of the most realistic contributions of the last decade to the fight against cancer.

The American Illustrated Medical Dictionary: A Complete Dictionary of the Terms Used in Medicine, Surgery, Dentistry, Pharmacy, Chemistry, Nursing, Veterinary Science, Biology, Medical Biography, Etc., with the Pronunciation, Derivation and Definition. By W. A. Newman Dorland, A.M., M.D., Lieutenant Colonel, Medical Reserve Corps, United States Army, member of the Committee on Nomenclature and Classification of Diseases of the American Medical Association; editor of the "American Pocket Medical Dictionary." Twenty-first edition. Price, $8 without thumb index; $8.50 with the thumb index. Pp. 1660, with 880 illustrations, including 233 portraits. Philadelphia and London: W. B. Saunders Company, 1947.

The first edition was published in 1900. The succeeding editions have represented well the steady growth of the medical vocabulary, and the present edition appears to cover as completely as can be expected the manifold additions to the vocabulary during the war and since. The standards of factual and typographic accuracy are well maintained. Many names of discarded proprietary medicines have been omitted, also some portraits. The only new portrait is that of Howard T. Ricketts, pathologist and pioneer investigator of rickettsial infections. The number of pages is about the same as in the previous edition. The definitions relating to cancer should be critically revised, beginning with the word "cancer" itself, which ·is defined as "any malignant tumor made up chiefly of epithelial cells. See carcinoma." In current usage, popular as well as technical, the term "cancer" includes sarcoma as well as carcinoma. "Alveolar carcinoma" and "mammary sarcoma" are examples of other cancer terms needing corrected definitions.

Le problème du cancer et son évolution recente (cancer et neuro-ergonologie). By Michel Mosinger, Institut d'Anatomie Pathologique de l'Université de Coïmbra (Portugal). Preface Prof. Gustave Roussy. Pp. 664, with 184 illustrations. Price, 1,000 francs. Paris: Masson & Cie, 1947.

The author reports a number of facts bearing on the etiology of cancer. The dominant idea is that the neurovegetative system plays an important direct or indirect role in the pathogeny of cancer. The cancerization of the normal cell is considered as the result of progressive phases: hyperplasia—benign tumor—cancer. The author presents the hypothesis that cancerization is essentially due to a disruption of the normal equilibrium of intracellular and extracellular substances. He distinguishes between carcinogens and carcinoinhibitors.

This appears to be a special issue (1944) of the Portugese journal *Arquivos de Anatomia Pathologica,* etc., presented in book form. The author is a French biologist who, at the time of writing, is director of the Institute of Pathologic Anatomy of Coimbra, Portugal. This probably explains the peculiar mixture of French with Portuguese text. (The latter language appears abruptly in page 30 and is kept through page 49, where it vanishes.)

The author reports his own work on 320 guinea pigs treated by injections of estradiol dipropionate and other products. As much as 243 pages are devoted to tedious enumeration of carcinogenic compounds with profuse text references, the majority of which are not listed in the bibliography. Sixty pages are devoted to a rather light description of multiple publications touching numerous subjects. The photographs are an example of the undesirable habit of using such means to balance a monotonous text; they are usually of poor quality.

The reader will be attracted by a section which purports to outline the perspective and organization that should be given to future research in cancer, but no one would escape the empty feeling that is left after reading it.

COLLECTED PAPERS OF THE MAYO CLINIC AND THE MAYO FOUNDATION. Edited by Richard M. Hewitt, M.D.; A. B. Nevling, M.D.; John R. Miner, Sc.D.; James B. Eckman, Ph.D.; Katharine Smith, B.A.; Carl M. Gambill, M.D., M.P.H., and Elizabeth L. Skafte, B.A. Volume 38, 1946. Pp. 915, with 149 illustrations. Price, $12.50. Philadelphia and London: W. B. Saunders Company, 1947.

REPORT OF THE HENRY PHIPPS INSTITUTE OF THE UNIVERSITY OF PENNSYLVANIA FOR THE PERIOD 1944-1946. Pp. 24. Philadelphia: Henry Phipps Institute, 1947.

INTERNAL MEDICINE IN GENERAL PRACTICE. By Robert Pratt McCombs, B.S., M.D., assistant professor of medicine and director of post-graduate teaching, Tufts College Medical School; senior attending physician, Joseph H. Pratt Diagnostic Hospital; specialist certified by the American Board of Internal Medicine. Second edition. Pp. 741, with 122 illustrations. Price $8. Philadelphia and London: W. B. Saunders Company, 1947.

ANNUAL REPORT FOR THE YEAR ENDED MAY 31, 1947. Issued by the National Foundation for Infantile Paralysis. Publication no. 68. Pp. 86, illustrated. New York: The National Foundation for Infantile Paralysis, 1947.

TRAUMATIC PROLIFERATIONS OF FIBROCARTILAGE WITH OSSIFICATION IN THE GENESIS OF SPONDYLITIS DEFORMANS AND MYOSITIS OSSIFICANS

EDWIN F. HIRSCH, M.D.

AND

JAMES W. RILEY, M.D.

CHICAGO

SPURS of bone arising on the bodies of the vertebrae with spondylitis deformans develop, according to many reports, because of stress applied to the vertebral column. The factors concerned with the growth of these adventitious bone tissues have been investigated, and excellent descriptions and summaries with large bibliographies have been published.[1] The current opinion of spondylitis deformans as reviewed and stated by Lang provides a considerable number of the basic concepts mentioned as background material in our report.

The term "spondylitis deformans" is used commonly in descriptions of these bone growths observed along the spinal column, although Schmorl preferred "spondylosis deformans" as more expressive of the nature of the disorder. The bone spurs appear on the anterior and lateral surfaces of the bodies of the vertebrae in consequence of changes in the elasticity of the intervertebral disks. These retrogressive changes in the tissues of the disks lead to softening, protrusion and even complete absorption. The posterior edges of the vertebrae, according to Schmorl and Junghanns,[1c] are not subject to stress such as occurs along the anterior edges, and hence spurs of bone do not develop there. In size the bone spurs range from small thickenings to those of considerable dimension. Beneke[1a] observed them mainly on the right side

1. (a) Beneke, R.: Beiträge zur wissenschaftlichen Medicin: Festschrift dargeboten den medicinischen Theilnehmern an der LXIX Versammlung Deutscher Naturforscher und Aerzte, Braunschwieg, Harald Bruhn, 1897, pp. 109-131. (b) Junghanns, H.: Arch. f. klin. Chir. **166**:121, 1931. (c) Schmorl, G., and Junghanns, H.: Die gesunde und kranke Wirbelsäule im Röntgenbild, Leipzig, Georg Thieme, 1932. (d) Schmorl, G.: Arch. f. klin. Chir. **172**:240, 1933. (e) Lang, F. J.: Arthritis deformans und Spondylitis deformans, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1934, vol. 9, pt. 2, pp. 265-368. Most of the pertinent literature is cited in this article.

of the lower thoracic and on the left side of the lumbar vertebrae. This, he explained, is due to the stress on the right upper extremity with various occupations which angulates the thoracic portion of the spinal column, with a concavity to the right and a corresponding lumbar scoliosis. Schmorl and Junghanns [1c] observed opposite relations in the spinal columns of those who were left handed.

The initial stages of bone spur formation appear in the third decade of life, occur about equally in men and women and depend on occupation. The incidence of the growths increases with age, so that after seventy years more than 90 per cent of all human spinal columns are reported to have them. The new growth of bone begins as a triangular extension from near the edge of the vertebral body. It does not arise at the edge, according to the studies by Schmorl and Junghanns, [1c] but from the body below the edge, at a level where in youth the vertebral body and the cartilaginous intervertebral disk are in contact. Here the anterior longitudinal ligament bends away and only a few fibers extend to the bone at the cartilage margins of the adjacent vertebrae and the intervertebral disk. Stress on the tissues of the periosteal ligament [1c,d] causes marginal growth at the level where the anterior longitudinal ligament again is attached firmly to the vertebral body. The firm union here between the marginal edge of the annulus fibrosus of the intervertebral disk and the marginal edge of the body of the vertebra is loosened by stress, the outer ring layers of the annulus fibrosus of the disk are compressed and the fiber bands are torn. Some growths occur, according to Schmorl and Junghanns, [1c] when margins of adjacent bodies rub together following the disappearance of the intervertebral disk.

Beneke [1a] and Lang [1e] expressed the belief that the bone tissues grow because a decrease in the elasticity of the ligament renders it impossible for a unilateral force to be distributed to all sides of the spinal column. The inelastic tissues transmit applied forces forward, predominantly in the direction originally received, and without absorbing the impact. Thus, more and more, the applied forces pass unchanged from one vertebral body to another, whereas under normal conditions these bodies are cushioned by the ligaments. The strengthening of certain bracing systems which follow the changes in the cushioning effect of the intervertebral disks involves all of the tissues participating. These are the cartilage margin of the intervertebral disk, the bone and adjacent marrow tissues and the periosteum.

The thin layer of hyaline cartilage in the intervertebral disk bordering the body of the vertebra is responsible for the physiologic growth in height of the body. This cartilage is in the center of the disk, and the margin has only isolated plates of cartilage in a fibrillar connective tissue, like tissues in many tendon insertions. These tendinous tissues are attached directly to the bone trabeculae of the vertebral body, while

the hyaline cartilage plate in the center closes the marrow spaces of the substantia spongiosa.

Lang [1e] stated that the changes in the intervertebral disk and the resulting failure to equalize the various stresses, especially at the periphery, cause a slow growth of fibrocartilaginous tissues, which ossify. The fibrocartilaginous tissues of the periosteum also may participate in the growth of these bone spurs. The cartilaginous tissues frequently have an increased cell content and resemble those seen at the epiphysial margins in youth. Similar tissues on the outer surface of the spurs consist mainly of fibrous stroma and cartilage and accordingly repeat phases of a physiologic growth of bone. The islets and strips of cartilage encased in widely separated trabeculae of the bone spurs in spondylitis deformans are substantial additional evidence of this enchondral and subchondral growth process. Beneke,[1n] as early as 1897, stated that islets of cartilage widely scattered in bone trabeculae are strong evidence for concluding that the bone has developed through a process of enchondral ossification, and he clearly presented in his report this concept of the enchondral bone formation in the origin of the spurs associated with spondylitis deformans.

Studies of the structure of the bone newly formed along the vertebral column in spondylitis deformans demonstrated to Schmorl, Beneke and Lang that the bone tissues arise through physiologic enchondral ossification of fibrocartilaginous tissues which have proliferated because of stress or trauma. These observations and conclusions are significant, because osseous tissues growing in muscles and in their tendinous insertions in consequence of trauma, dislocation, simple fracture of a bone or a minor injury have been found by Hirsch and Morgan [2] to pass through a similar, although perhaps more rapid, cycle of fibrocartilage proliferation and enchondral ossification into the quiescent bone stage. This disorder has been described under such terms as "traumatic myositis ossificans," "parosteal callus" or others appropriately descriptive. Many theories,[3] some highly improbable, have been proposed to explain the development of bone in traumatic myositis ossificans. Meyenburg [4] stated that the nature and the cause of this disorder had not been clarified. He classified the lesions into (1) those following a single severe injury, such as a blow, a dislocation of a joint or a fracture of a bone, and (2) those appearing after repeated small traumas that occur to muscles in various forms of physical activities. He concluded from a review of the structures observed in these lesions that the bone develops partly from fibrous tissues and partly from cartilage.

2. Hirsch, E. F., and Morgan, R. H.: Arch. Surg. **39**:824, 1939.

3. Carey, E. J.: Arch. Surg. **82**:592, 1924.

4. von Meyenburg, H.: Die quergestreifte Muskulatur, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1929, vol. 9, pt. 1, pp. 363-372.

The early phases of the lesions, he stated, have an excess of cartilage; the later, a predominance of bone. After a lengthy comment on various published opinions, Meyenburg concluded that the bone in traumatic myositis ossificans is a product of metaplasia in which muscle stroma definitely participates, and perhaps also the muscle fibers. Hirsch and Morgan obtained tissues from the lesions of 11 patients with traumatic myositis ossificans. Some of these were in the so-called "green" stage, and these authors were able to trace the evolution of bone through cellular fibrocartilage or cartilaginous tissues until, in the final stages, only small remnants of cartilage remained in the bone trabeculae. An inquiry into a matrix of fibrocartilaginous tissue which could give rise to the initial fibrocartilage proliferation led them to studies of tendon insertions. These reaffirmed statements in histologic descriptions of animal tissues that fibrocartilage is found commonly in tendon insertions. Hirsch and Morgan reasoned, accordingly, that in traumatic myositis ossificans bone develops through ossification of fibrocartilaginous tissues that have proliferated following a traumatic injury of the tendon insertions. After this reactive proliferation of the fibrocartilaginous matrix, the physiologic sequence of enchondral ossification occurs.

The comprehensive studies of Schmorl, Beneke and Lang of the bone tissues found with spondylitis deformans and their conclusion that trauma causes fibrocartilaginous tissues to proliferate and subsequently pass through enchondral ossification bring this disorder into a common pattern of causation and structure with myositis ossificans, in which enchondral ossification was observed by Hirsch and Morgan, who likewise concluded that fibrocartilaginous tissues stimulated to growth by injury or stresses are the essential, the basic tissue constituents of this disorder. In spondylitis deformans the evolution of the bone elements is much slower than in traumatic myositis ossificans. In the former the effects of small repeated traumatic stresses gradually produce the new tissues, whereas in the latter a large single trauma or multiple smaller traumas initiate the process and cause a marked reactive proliferation of fibrocartilaginous tissues, which ossify and in a comparatively short time reach the quiescent stage.

To compare the bone tissues of myositis ossificans with those of spondylitis deformans and to reinvestigate the evidence of enchondral bone formation in the latter, 23 tissues taken from vertebral bodies with and without these changes were examined. The bone tissues were fixed in Zenker's solution, decalcified and embedded in paraffin or celloidin (a concentrated preparation of pyroxylin). The sections were stained with hematoxylin and eosin. Tissues from vertebrae without the bone growths included tendon insertions, and in these were small masses of fibrocartilage (fig. 1). The sections of the new bone growths with attached soft tissues had, at the junction of the soft parts and the bone, cartilage and fibrocartilage in varying amounts. The

Fig. 1.—Photomicrograph illustrating fibrocartilaginous tissues in the tendons where these insert into the bodies of the vertebrae. × 198

Fig 2 —Photomicrograph illustrating residues of cartilage in the trabeculae of the bone spurs that develop with spondylitis deformans × 198.

larger exostoses had only small amounts of hyaline and fibrocartilaginous tissues; the smaller, perhaps more actively progressing, exostoses had much larger amounts. The trabeculae of bone in these, also, had irregular residues of cartilage (fig. 2). Such residues of cartilage in many of the trabeculae of bone growths of myositis ossificans were considered especially significant by Hirsch and Morgan as evidence of enchondral ossification nearing completion.

The trabeculae of the exostoses of the spinal column with spondylitis deformans thus bear evidence, as others have described and stated, that they arise through enchondral ossification. The evolution occurs over a span of many years, with minimal accretions of bone, until presumably adequate stress-resistant tissues are formed. With traumatic myositis ossificans the bone growth is not compensatory to a continuous stress but is rather a spurious fibrocartilaginous tissue reaction to a traumatic injury, eventually becoming differentiated bone as an end stage of physiologic enchondral ossification.

The observations and conclusions of others, supplemented by our own, that the exostoses of spondylitis deformans develop through enchondral ossification of fibrocartilaginous tissues stimulated to growth by stress are comparable with the descriptions and conclusions of Hirsch and Morgan concerning the bone formed in traumatic myositis ossificans. These studies and conclusions developed independently and by different authors reveal enchondral ossification as a normal sequence of traumatic proliferations of fibrocartilage in two disorders that have seemed to be entirely unrelated.

SUMMARY

Bone spurs develop along the vertebral column in spondylitis deformans through enchondral ossification of proliferated fibrocartilage, activated to growth by the trauma of stress.

The lesions of traumatic myositis ossificans evolve through a similar process of enchondral ossification in proliferated fibrocartilage.

The bone spurs of spondylitis deformans develop along the vertebral column, in a sense, to compensate and strengthen ligaments weakened by continuous stress at their points of insertion. The bone growths of traumatic myositis ossificans are spurious growths, occurring in tendons or ligaments traumatically injured at their points of insertion.

The fibrocartilaginous tissues stimulated to proliferate by injury are normal constitutents of ligaments and tendons where these attach to bone.

Accordingly, enchondral ossification occurs as a normal sequence of traumatic proliferations of fibrocartilage in the two disorders, spondylitis deformans and traumatic myositis ossificans, which have seemed to be entirely unrelated.

Trauma is the accepted cause of each.

NEUROGENESIS OBSERVED IN A MIXED GRAWITZ-WILMS TUMOR

CHARLES G. De MUYLDER, M.D.

LOUVAIN, BELGIUM

THE LEFT kidney was removed from a boy 3 years of age because of tumor. A mass like a goose egg was seen partly invading the inferior pole; this mass contained numerous little cysts (fig. 1).

MICROSCOPIC OBSERVATIONS

Several pieces of the tumor and of the kidney were taken for biopsy. Various technics were used: Masson's trichrome method with aniline blue, Van Gieson's method, Foote's silver impregnation for reticulin and Rogers' silver impregnation for the nervous system. Frozen sections were also made and stained with sudan III for lipids.

On microscopic examination the tumor was very polymorphous; several fields, indeed, were highly characteristic of different types of neoplasm.

A typical hypernephroma (Grawitz' tumor) was present (fig. 2). The cysts (fig. 3) were mainly connected with that type; they formed spherical cavities lined by one layer of a neoplastic epithelium. This epithelium was sometimes very anaplastic in appearance and invaded the deeper structures; transitional foamy cells were observed, but this was not the rule; usually the anaplastic cells became pear shaped (9 by 36 microns) and bulged into the lumen of the cyst: their cytoplasm was granular and was stained by both acid and basic dyes; the nucleus was elongated (20 by 6 microns) and showed grossly irregular chromatin. The basis of these cells was ill defined and vanished gradually between the underlying structures; it was sometimes characterized by a dark-staining expansion originating from the cell body in the vicinity of the nucleus and running toward the periphery in a translucent sheath.

Also present was a sarcomatous area with very dense collections of small cells and tubular formations (Wilms' tumor); the adenosarcomatous masses were separated by a loose mesenchyma. Fibrillar networks running between the cells of the blastema were apparent in several places.

The fibrillar bundles extending through the adenosarcomatous areas and along the new-formed tubules were nervous: Their structure did not correspond to anything like collagen fibers or any form of connective tissue; the fine network was stained red by Masson's trichrome method, whereas collagen is stained blue (aniline blue); by Van Gieson's method it stained yellow, whereas collagen stains red; in Foote's impregnation for reticulin the silver left this network quite

---

untouched; finally, Rogers' silver impregnation technic for the nervous system
electively stained these fibers black; this impregnation revealed at the same time
the neuroblastic nature of several pear-shaped cells by showing neurofibrils in
their cytoplasm (fig. 4).

Fig. 1.—Cut specimen showing the normal superior pole of the left kidney, the
tumor invading the inferior pole, with multiple cysts, and the sites of removal of
several portions of the specimen for biopsy.

Fig. 2.—Photomicrograph: Both hypernephromatous (*H*) and adenosarcoma-
tous (*S*) conditions are present; there is no well defined tubular structure in this
field. (Masson's trichrome stain.)

Fig. 3.—Photomicrograph. Anaplastic cells bordering a neoplastic cyst.
(Masson's trichrome stain.)

The basic element of the hypernephroma was not always as regular as described previously; this became evident on frozen sections when the typical distribution of fatty droplets appeared in cells which the more distant they were from the center of the area considered hypernephromatous the more different they were from the original type in shape: they became spindle shaped, although they retained their fatty constituent. Some of these spindle-shaped cells underwent another transformation; i.e., they showed one or two very long expansions and became unipolar or bipolar cells; with the routine trichrome stain, the cytoplasm of such cells showed besides the empty vacuoles (which corresponded to lipids) a fine reddish fibrillar brush entering the main expansion, where the fibrils became pressed together and gradually lost their individuality; usually one fibril was more obvious than its neighbors, staining black with hematoxylin; it could thus be followed as it coursed around the vacuoles up to the vicinity of the nucleus. Wherever these expansions were gathered in a bundle the disposition was such that one could not escape the impression that they formed a nerve tract. The various staining tests entirely confirmed this view: The fibers were never blue by Masson's technics, never red by Van Gieson's method; they were never impregnated by Foote's silver method but were electively impregnated by Rogers' silver method for nerve fibers (fig. 5). In mature cells one could see the typical neurofibrils twisting around the not less typical vacuoles; in younger cells the neurofibrils were well impregnated at the root of the expansion only. These observations would suffice to prove the nervous connections of the hypernephroma but I had the good fortune to make several other observations which led to the same conclusion.

As stated, the main cysts were connected with the hypernephroma, and sometimes their epithelium showed a foamy cytoplasm; here and there this neoplastic epithelium broke through the basal membrane and budded in the underlying structures. An instance must be described here: A cyst with a single layer of foamy epithelium (quite similar to the hypernephroma) produced by budding a large quantity of cells which, one by one, invaded the depth of the tumor, where they underwent typical changes; stained by the routine method, they appeared first of all to be spindle shaped, with a dark red homogeneous body; the deeper they got in the tumor, the larger they became; their cytoplasm got darker and lost its homogeneity to take the appearance of an ill defined network; expansions appeared in the majority of cases; Rogers' method showed the extraordinary affinity of these cells for silver nitrate, also the nervous nature of the cytoplasmic network as well as of the expansions. These cells were sometimes very anaplastic (fig. 6). Another form of neurogenesis was thus present, originating from the hypernephromatous cysts and invading the deeper masses of the Grawitz tumor.

To support the fundamental fact of nervous potentiality, many more documents are available. I shall describe here just one more picture which is of special importance and leads to the next point: the true origin of the nerve elements.

On serial sections I followed a band of tissue apparently enclosed in a collagenous sheath and showing several interesting features; this band consisted of small pear-shaped cells, each with a thin layer of dark red cytoplasm surrounding an oval nucleus; the first remarkable thing was that the sheath instead of being continuous showed several holes occupied by the blind extremities of such tubules as were produced by the Wilms tumor; the basal membrane of the tubules was lacking in the part which was inside the sheath; this disposition reenforced the intimate contact between the tubular and the piriform cells, the two of them not

only being in close relationship but also looking very similar indeed. The second feature was the appearance in the enclosed mass of sketches of balls and tubules morphologically indistinguishable from the peripheral nephrogenous tubules. These two points prove the essential connection linking the nephrogenous blastema to the pear-shaped cells through the tubular formations; this view is confirmed by the existence of all intermediate stages in the series of sections. The third point
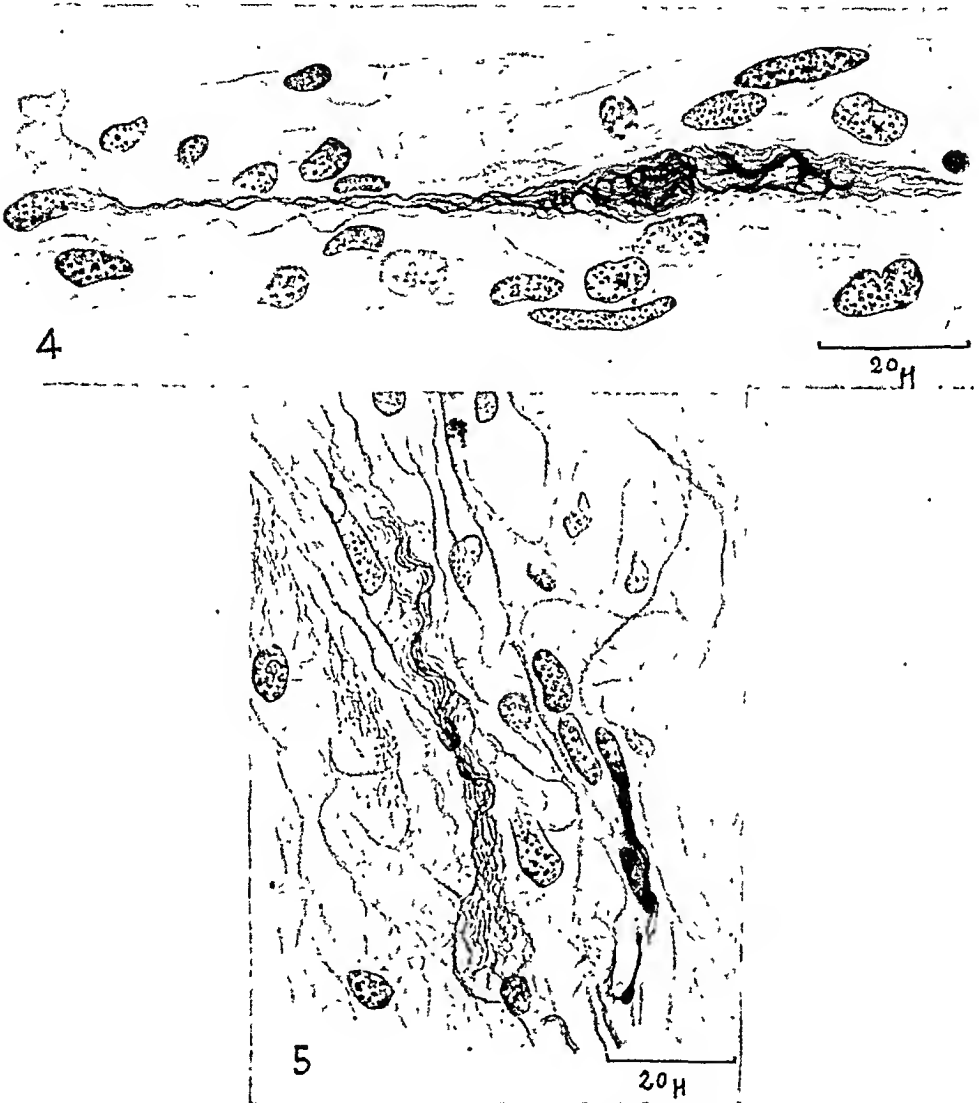
Fig. 4.—Drawing: Originating from the adenosarcoma, a cell is seen filled with silver-impregnated fibrils; in this cell vacuoles make their appearance between the fibrils (Rogers' silver impregnation.).

Fig. 5.—Drawing: Typical neurohypernephromatous cell. (Rogers' silver impregnation.)

concerns the evolution of some of the pear-shaped cells; their thin extremity grew longer and stained darker, becoming a real expansion, as described previously; at its insertion on the main cytoplasmic body the dark expansion seemed to divide

6
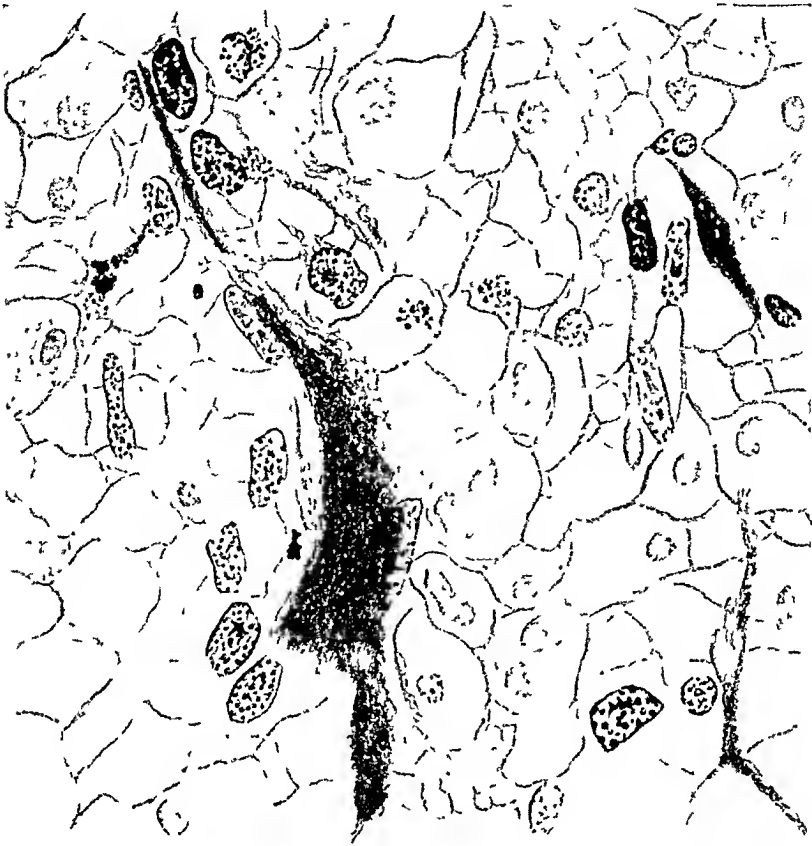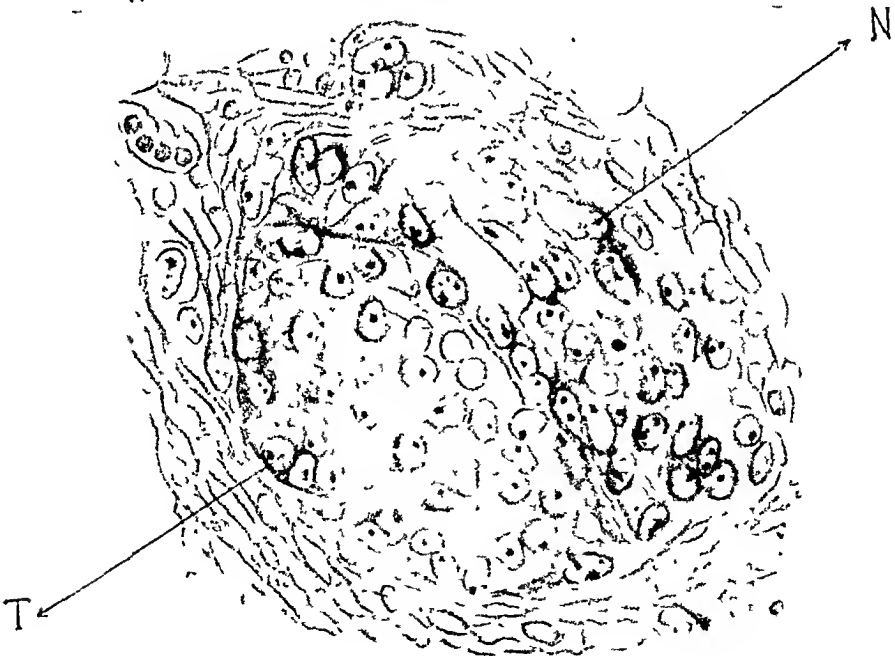
7

Fig. 6.—Drawing: Anaplastic cells filled with neurofibrils. (Rogers' silver impregnation.)

Fig. 7.—Neoplastic invasion (N) of a capsular space; the glomerular tuft (T) is shrinking. (Masson's trichrome stain)

into two tiny bands which ran in the outer area of the cytoplasm just inside the cellular membrane, where they formed a kind of exoplasm; they enclosed a much paler endoplasm, roughly triangular in shape. The next step might be the development of another expansion at the opposite pole (bipolar cells) or only an enlargement of the unipolar cell; when this took place, more details became apparent: Very often a third dark band was then visible, extending from the bifurcation just referred to, as a supplementary branch, in the direction of the nucleus; in some instances its course became distorted by typical round and clear vacuoles, appearing in the endoplasm in the same way as in the hypernephroma. The end result was a neurohypernephromatous cell just like the cells I described on page 453. Confirmation of this was given by silver impregnation; the modified cells showed an affinity for silver nitrate (Rogers' method) which was in close relationship to the degree of fibrillar differentiation—the more mature cells showed typical black neurofibrils, while the younger elements displayed only a diffuse grayish tint.

The Grawitz tumor and the Wilms tumor were fundamentally linked by a neurogenic potentiality; both neoplastic types derived from the same nervous stem, the Grawitz tumor being differentiated and the Wilms tumor embryonic; that both are also truly nephrogenic will be explained in the next paragraph.

Examining, on serial sections, the transition between the kidney and the tumor I had the good fortune to find, next to fields displaying mostly sclerosis, numerous other areas demonstrating a process of neoplastic degeneration; this process originated in a zone where the normal nephron was reduced to a few remnants: the glomerulus with a short appendix of proximal convoluted tubule, and, at the other end of Bowman's capsule, this very segment of the distal convoluted tubule, which was characterized by the presence of a "macula densa" in the angle of the afferent and efferent vessels. The closed segment of pars convoluta II became cystic, and its epithelium underwent anaplastic changes giving rise to exactly the same epithelium as was found in many cystic conditions of the hypernephroma described on page 452. In the meantime, the glomerular tuft degenerated and shrank, but the outer epithelium of Bowman's capsule underwent a striking change; the process started exactly at the neck of pars convoluta I, and the neoplastic disease spread all over Bowman's capsule; as a result, the capsular space was bordered (sometimes obliterated) by a neoplastic epithelium which was the same as the epithelium of the cystic pars convoluta II (fig. 7); the glomerulus becoming cystic after the disappearance of the vascular tuft would be indistinguishable from the other cysts of apparently different origin. In the next step the cystic epithelium proliferated through the basal membrane, forming small cellular cords invading the underlying connective tissue and eventually starting the whole process over again in pure neoplastic form. This was an adenosarcomatous type of condition with which the hypernephromatous type mixed gradually by every possible transitional form. The true nephrogenic origin of these otherwise *neurogenic* tumors was thus evident; neurogenic elements must have been present (*a*) in pars convoluta II where it lies on the vascular hilus of the glomerulus (site of the "macula densa") (Zimmermann [1]; Goormaghtigh [2]) and (*b*) in the origin of pars convoluta I, at the neck of the glomerulus. The full description of the observations on the nervous constituents of the nephron will be published separately.

1. Zimmermann, K. W.: Ztschr. f. mikr.-anat. Forsch. **32:**176, 1933.

2. Goormaghtigh, N.: La fonction endocrine des artérioles rénales, Louvain, Belgium, R. Fonteyn, 1941.

COMMENT

The facts reported confirm Masson's [3] description of the embryonal adenosarcoma of the kidney; they reveal also the true nature of the typical hypernephroma. This nature has been discussed by Grawitz, Birch-Hirschfeld, Stoerk, Sudeck and others. Recently Riopelle [4] reviewed the subject of hypernephroma but did not make any reference to neurogenesis. To my knowledge, nobody has ever brought forward any substantial evidence in favor of the neurogenic signification of the hypernephroma. It seems that considering the hypernephroma as a differentiated tumor of the neurogenic constituent of the kidney would explain the difficulty experienced by the authors in trying to classify it. It is of course a true renal tumor, but, more than that, it is a nervous tumor. The only possible origin of such a nervous constituent being the neural crest, it seems logical to ascribe it to this structure. One does not overlook a local origin from the mesenchyme, but one does not have any fact to support this hypothesis; on the other hand, one has some evidence in favor of the first one: The study of serial sections from embryonic material (human embryos and pig and chicken embryos) suggests the existence of a loosening of the neural crest in the lumbar mesenchyme which might give rise to an ectomesenchyme (see also Van Campenhout [5]). This contribution of the neural crest to the constitution of the lumbar mesenchyme might afford a unifying understanding of the embryogenesis and the pathology of the various organs which develop in that region; this ectomesenchyme might participate in the development of the adrenal cortex, the renal parenchyma and the interstitial elements of the gonads. Indeed, all these organs are liable to the development of hypernephroma, which has been considered so far as originating from heterotopic adrenal tissue; according to the suggestion made here, tumors of this type would represent a common histogenetic potentiality deriving from a common constituent: the ectomesenchyme. This view is in accord with Schiller's [6] conclusions on the extension of the hypernephromatous potentiality along the urogenital fold. It meets some aspects of the hypothesis of Horta [7] that the renal hypernephroma, being a tumor of the same class as certain neoplasms arising from near-by endocrine organs (cortex of the adrenal gland, ovary, testis), might be a tumor of the kidney. So far I have not found any relationship between this hypernephroma and the

3. Masson, P.: Am. J. Cancer **33**:1, 1938.

4. Riopelle, J. L.: Rev. canad. de biol. **4**:40 and 66, 1945.

5. Van Campenhout, E.: Arch. de biol., Paris **42**:479, 1931: **48**:611, 1937.

6. Schiller, W.: Arch. Path. **30**:879, 1942.

7. Horta, J. S.: Arch. españ. urol. **11**:115, 1945; abstracted. Internat. Abstr. Surg. **82**:309, 1946.

cells first described by Ruyter [8] and studied principally by Goormaghtigh [2] in cases of high blood pressure.

SUMMARY

The hypernephroma (Grawitz' tumor), as well as Wilms' tumor, has neurogenic potentialities; the neurogenic constituent of both neoplasms derives from normal components of the renal tubules; these components probably represent the contribution of the neural crest to the lateral lumbar mesenchyme.

8. Ruyter, J. N. C.: Ztschr. f. Zellforsch. u. mikr. Anat. 2:242, 1925.

TISSUE CHANGES IN FUNGOUS DISEASE

ROGER DENIO BAKER, M.D.

BIRMINGHAM, ALA.

FUNGOUS infections are often spoken of as though the tissue changes which they cause were stereotyped. I wish to analyze this point of view and to show that there is, on the contrary, much variation. Observations made on a relatively large series of cases of all types of fungous infections show that there are many differences in the tissue changes. Even acute inflammation may be present. In the more chronic infections there is much variation in the kind of chronic reaction. Moreover, it is possible to group the fungous infections according to types of tissue changes. Reemphasis is given to the suppurative aspect of the mycoses.

An unusually good opportunity to make a study of this kind was provided by the Fungus Disease Registry, in which there had accumulated several examples of all the usual mycoses. The nomenclature used is that presented in the "Manual of Clinical Mycology." [1]

The tissue changes subject to tabulation were those of inflammation, *necrosis and repair. The inflammatory reaction was subdivided into* the features of suppuration, the macrophage reaction and the giant cell reaction. Suppuration was defined as an accumulation of neutrophils together with a liquefaction of tissue to form an abscess. Some attention was given to other types of cells which might be intermingled with neutrophils but in smaller proportions, such as eosinophils, lymphocytes or plasma cells. The macrophages and giant cells are probably to be thought of as an integral part of the inflammatory response, but they may also be considered as phagocytic cells quite apart from this participation. Caseous necrosis was rather like that observed in tuberculosis. It showed the essential features of necrosis in the form of

1. Conant, N. F.; Martin, D. S.; Smith, D. T.; Baker, R. D., and Callaway, J. L.: Manual of Clinical Mycology, ed. 1, Philadelphia, W. B. Saunders Company, 1944.

karyolysis with varying amounts of karyorrhexis and pyknosis. Of course, necrotic cells were present in areas of suppuration, but caseous necrosis was tabulated only when truly of caseous type. Fibrosis was included in the tabulation whenever a fibroblastic repair process, with fibroblasts or collagenous connective tissue, was noted.

The use of the terms "granuloma" and "granulomatous" has been avoided, since there is variation in the usage of these terms and since the fundamental components can be mentioned specifically.

The changes occurring in the deep fungous infections are considered first, because more information is available concerning these changes than those of the superficial infections.

The tissue alterations could be grouped in five categories with respect to the features of inflammation, necrosis and fibrosis which have been previously mentioned, i. e., suppuration, macrophages, giant cells, caseous necrosis and fibrosis.

GROUPING ACCORDING TO TYPES OF TISSUE CHANGES

Group 1:   All Features (Suppuration, Macrophages, Giant Cells, Caseous Necrosis and Fibrosis) Often Present.—Four types of fungous infections are found in this group (table 1).

The tissue reactions in human blastomycosis (North American) have been given special attention previously.[2] In that report it was pointed out that in the 23 cases studied, polymorphonuclear abscesses were always present. Giant cells were always present; caseation was present in the generalized and in many of the deep infections but was not noted in the cutaneous ones. Large mononuclear cells were by no means as prominent as neutrophils, but they were frequently observed, as were eosinophils, lymphocytes and plasma cells. Fibrosis was frequently prominent. In many lesions fibrosis occurring about abscesses had developed a heavy collagenous component. It was concluded that human blastomycosis was primarily pyogenic, with prominence of polymorphonuclear neutrophils. A few lesions in some of the cases, especially in the systemic group, closely resembled the lesions of tuberculosis.

For the present study no additional tabulation of North American blastomycosis was carried out.

A summary of 5 cases of South American blastomycosis showed the following changes: suppuration, plus or minus; macrophages, 1 plus; giant cells, 1 plus; caseous necrosis, 1 plus; fibrosis, 2 plus. Lymphocytes were prominent in a number of cases. (See figure 43 in the "Manual of Medical Mycology" for the neutrophilic reaction and figure 44 for the giant cell reaction.[1])

Coccidioidomycosis was represented by 8 cases, as shown in table 1.

---

2. Baker, R. D.: Am. J. Path. **18**:479, 1942.

Giant cells and fibrosis were present in all the cases of coccidioido-mycosis. In 3 cases suppuration was absent. This does not necessarily mean that the primary reacting cell had not been the neutrophil but rather that the process had reached the stage of fibrosis.

In a study of 95 cases of disseminated coccidioidomycosis Forbus and Bestebreurtje[3] show the presence of all the changes indicated in my group 1. They state: "The histologic character of the inflammatory reaction varies greatly in any given lesion, and from lesion to lesion in the same individual, and appears to be determined chiefly by the number and the developmental stage of the organisms present in the lesion." They noted a tendency for suppuration to predominate where free endospores occurred, and for macrophages and giant cells to be numerous about spores of greater maturity.

TABLE 1.—*Fungous Infections with All Features Often Present (Suppuration, Macrophages, Giant Cells, Caseous Necrosis and Fibrosis)*

North American Blastomycosis	South American Blastomycosis	Coccidioidomycosis			Sporotrichosis
		Microscopic Characteristics of Coccidioidomycosis			
Case	Suppuration	Macrophages	Giant Cells	Caseous Necrosis	Fibrosis *
1......................	....	++++	+	++	+
2......................	....	....	++	....	++++
3......................	+	+	+++	+	++++
4......................	++	....	+	++	+++
5......................	....	....	++	....	+++
6......................	+	++	++	++	++
7......................	+	+	++	+++	+
8......................	+++	....	++	....	++

* Lymphocytes and plasma cells were often noted in the fibrous tissue.

The tabulation of sporotrichosis was taken from 3 cases. In summary they showed: suppuration, 1 plus; macrophages, 2 plus; giant cells, 2 plus; caseation, 3 plus; fibrosis, 3 plus. Suppuration occurred in 2 of the 3 cases and caseous necrosis in all 3. The lesions of sporotrichosis are well described as "gumma-like nodules, ulcers and abscesses" by Mackie, Hunter and Worth.[4] Caseous necrosis would seem to be rather conspicuous in sporotrichosis.

Group 2: All Features Except Caseous Necrosis Often Present.— The types of fungous infections included in table 2 show suppuration. macrophages, giant cells and fibrosis, but caseous necrosis is not noted. Actinomycosis and nocardiosis, caused by the Pseudomycetes, belong here, as does maduromycosis, caused by the Eumycetes. Chromoblastomycosis also belongs here.

3. Forbus, W. D., and Bestebreurtje, A. D.: Mil. Surgeon 99:653, 1946.

4. Mackie, T. T.; Hunter, G. W., III, and Worth, C. B.: A Manual of Tropical Medicine, Philadelphia, W. B. Saunders Company. 1945.

In a group of 6 cases of actinomycosis, suppuration was noted as 4 plus, macrophagic response as 2 plus, giant cell response as 1 plus and fibroblastic response as 4 plus. Caseous necrosis was noted in 1 of the 6 cases, indicating that this change may occur, though it is not characteristic. Giant cell reaction was noted in 2 of 6 cases. The purulent nature of actinomycosis is well known and manifests itself by large amounts of pus draining from the sinuses. The giant cell reaction of actinomycosis is usually not marked, but occasionally giant cells are found in apposition with the granules. The macrophages are often laden with fat. The lymphocytic and plasma cell infiltration is variable. It often occurs in the scar tissue.

The tissue responses in maduromycosis (table 2) are almost identical with those in actinomycosis and nocardiosis. Moreover, plasma cells and lymphocytes are often present.

TABLE 2.—*Fungous Infections with All Features Except Caseous Necrosis Often Present (Suppuration, Macrophages, Giant Cells and Fibrosis)*

Actinomycosis Nocardiosis		Maduromycosis		Chromoblastomycosis	
		Microscopic Characteristics of Maduromycosis			
Case	Suppuration	Macrophages	Giant Cells	Caseous Necrosis	Fibrosis *
1 .. .	++	++	....	....	+++
2..	++	++	+	. .. ...	+++
3. . .	++	++++	++	....	++
4 . .	+++	++	....	....	++
5. ...	++++	+	....	.	++
6.	++++	+	+	....	++++

* Plasma cells and lymphocytes were often present.

Chromoblastomycosis, 4 cases, showed suppuration, macrophages, giant cells and fibrosis in all and caseous necrosis in none. Plasma cells were prominent in the fibrous tissue in 2 cases. Suppuration was prominent in 2 and minimal in 2.

Group 3: Suppuration Usually Absent.—Table 3 shows two types of fungous infection in which suppuration is usually absent.

Sections were examined in 4 cases of histoplasmosis, and suppuration was noted in none. Probably cases are on record in which mild neutrophilic response has been seen, but it is not characteristic of the disease. Macrophages or reticuloendothelial phagocytosis was prominent in all. Giant cells were noted in 2 cases, fibrosis in 1 and caseous necrosis in 2. Generalized histoplasmosis frequently occurs purely as a cytomycosis, the organisms having been phagocytosed by the cells of the reticuloendothelial system, particularly in the liver, the spleen, the lymph nodes and the bone marrow. It is often like a storage disease rather than an inflammatory process. Where there has been conglomer-

ation of macrophages or reticuloendothelial cells containing parasites, there tends to be caseous necrosis. Fibrosis was noted in an ulcer of the tongue.

Of 7 cases of cryptococcosis, suppuration was absent in 6. In the seventh case, in which there was a lesion of the shoulder, suppuration was noted as 2 plus. Thus suppuration may be present, but usually is absent. One case was observed in which there appeared to be no reaction of any sort except for the presence of a minimal number of lymphocytes. Giant cells were present in the other 6 cases, and fibrosis was noted in 5 cases. Lymphocytes occurred in the fibrous tissue. There appeared to be some relationship between the duration of the infection and the formation of giant cells and fibrosis. Caseous necrosis was noted in 2 cases.

Group 4: Acute Inflammation and Necrosis.—Table 4 shows two types of fungous infection in which acute inflammation and necrosis were noted.

TABLE 3.—*Fungous Infections in which Suppuration Is usually Absent*

Histoplasmosis	Cryptococcosis

TABLE 4.—*Fungous Infections with Acute Inflammation and Necrosis Present (Macrophages, Giant Cells and Fibrosis Usually Absent)*

Mucormycosis	Aspergillosis

Sections obtained in 2 cases of probable mucormycosis of the brain and of the nose, with death after an illness of only several days, showed neutrophils in both cases and caseous necrosis in one. No macrophages or giant cells were present, and there was no fibrosis.

In several cases of aspergillosis of the ear there was acute inflammatory reaction about the filaments and spores. Other types of reaction occur in aspergillosis. For example, suppurative infarcts of the lung and abscesses of the vitreous of the eye have been reported.[5]

Many other fungous infections would certainly be attended with acute inflammation or with necrosis if encountered at an early stage of invasion. Mucormycosis, however, is an especially good example of a fungous disease in which the duration of the process is so short that there is time only for acute inflammation and necrosis to develop before death occurs.

5. Ash, J. E., and Spitz, S.: Pathology of Tropical Diseases: An Atlas, Philadelphia, W. B. Saunders Company, 1945.

Group 5: No Reaction, or Chronic Inflammation.—Some fungous infections are attended with no inflammatory reaction at all. This is true when infection of a nonvascular structure occurs, such as the skin, the hair or the nails. These same organisms, on occasion, may produce inflammation of deeper tissues. Moniliasis may occur in a similar fashion and produce the same types of reaction.

The histologic change in this group may be that of chronic inflammation without suppuration, giant cells or fibrosis. As is clear from the clinical course, there must be a stage of acute inflammation. This is not usually observed, because of the difficulty of obtaining histologic material at this stage. In some cases in which the tissues are deeply penetrated by organisms of the dermatophytes, suppuration, giant cells and fibrosis do occur, as in sycosis barbae.

Moniliasis is a fungous infection which tends to grow on surfaces, such as those of the mouth, the esophagus and the vagina. There may be extensive chronic inflammation, but the chief part of the lesion is often the mycelium itself. In rare cases of deep Candida albicans

TABLE 5.—*Fungous Infections with No Reaction or Chronic Inflammation (Suppuration, Giant Cells and Fibrosis Rare)*

Dermatomycoses of skin, hair, nails	Moniliasis

infection of the body, all of the tissue reactions of group 1 have been noted.[1]

It is possible that allergic reactions contribute to the tissue changes in many of the mycoses. The dermatophytids appear to be primarily of this sort. They would be classified in group 5, occasionally in group 4.

COMMENT

Certain correlations can be made between the tissue changes and the following factors:

1. Fungi as foreign bodies of large size. The fungi are of large size and may exert their effect partly because they are foreign bodies. Around a splinter of wood in the tissues, for example, there are neutrophils at first, giant cells next, and finally fibrosis.

2. Location of fungus. This factor may have great influence on the type of reaction. Thus growth on hair, epidermis or nails produces no reaction and may represent an infection without inflammation. Growth deep in the epidermis may produce acute or chronic, so-called nonspecific, inflammation. Growth of any of the fungi deep in the tissues may produce suppuration or caseous necrosis, but not always. The lack of caseous necrosis in some infections may be due to the fact that necrosis is occurring so slowly that no large areas are produced.

Liquefaction advances rapidly enough to prevent the maintenance of coagulation necrosis. It is an old observation that liquefaction occurs chiefly in those areas where the neutrophils are numerous.

3. Endotoxins and the chemical constitution of the organisms. The fungi· lack the strong toxic effect which certain bacteria have, such as the streptococcus and the organism of diphtheria. However, when fungi disintegrate, they undoubtedly liberate endotoxin. Relatively little convincing information is present on the chemical constitution of fungi in relation to the type of tissue change. While blastomycetic phosphatide repeatedly injected intraperitoneally into mice caused cells of the monocytic series to respond, and single intraperitoneal injections of. blastomycetic polysaccharide produced acute peritonitis in rabbits, there is question of the significance of these responses because of the time element and because of the type of animal used.[6]

4. Allergy. The role of allergy in the tissue change is difficult to evaluate. The allergic state is one in which the body is more sensitive to the endotoxins of the fungus than is the uninfected body. This is shown in tissues by more rapid and extensive necrosis and inflammation. Caseous necrosis may have some relationship to the development of an allergic response, but other factors, such as the necrosis of masses of parasite-containing macrophages caused by enzyme activity, may be equally important. The widespread necrosis of human sporotrichosis in the absence of demonstrable organisms speaks in favor of allergy.

5. Chronicity of infection. This factor is of great importance in the production of the changes seen in the deep infections. Suppuration continues because organisms persist and continually die off; macrophages and giant cells gather, and scar tissue forms. Organisms frequently survive and even proliferate within macrophages and giant cells.

From the observations of this report it is evident that no one tissue change is exclusively characteristic of, or pathognomonic of, fungous infection. Essentially all of the usual inflammatory and repair processes, and also necrosis, may occur. In the recognition or diagnosis of fungous infections some aid is provided by the type of tissue reaction. For example, chronic abscess formation should suggest the possibility of fungous infection. Caseous necrosis should also suggest fungous infection, but it is not as characteristic a finding. Giant cells suggest fungous infection but this may not be present.

If granulomatous inflammation is defined chiefly on the basis of participation of clusters of macrophages and giant cells, it seems clear that other features, such as suppuration and fibrosis, are equally characteristic of the fungous infections. If one thinks of granulomatous tissue as characterized by a focal type of reaction, such as the tubercle,

---

6. Baker, R. D.: Am. J. Path. **18**:463. 1942.

then some of the fungous infections are of this type; but the tubercles of fungous disease tend to have purulent centers in contrast to those of tuberculosis.

SUMMARY

When the microscopic appearances of fungous diseases were tabulated with respect to the degree of suppuration, macrophages, giant cells, caseous necrosis and fibrosis, the following observations were made: 1. Several of the deep fungous infections, such as blastomycosis (North and South American), coccidioidomycosis and sporotrichosis, show all of these tissue changes. 2. Others of the deep infections, such as actinomycosis, nocardiosis, maduromycosis and chromoblastomycosis, show all of these changes except caseous necrosis, as a rule. 3. A few of the deep mycoses are not attended with suppuration. 4. Mycoses may run their entire course with only acute necrosis or acute inflammation. 5. The superficial fungous infections often have no inflammatory response. On occasion they may have an acute or a chronic inflammatory response, or even less commonly, all of the responses.

It is concluded that chronic suppuration with fibrosis is probably the most general tissue change in deep fungous infections and that the neutrophil is more usually the primary reacting cell. In some instances, however, the macrophage or the giant cell may be the primary reacting cell. Factors which may be responsible for the tissue changes of fungous infections are the following: (1) the large size of the organism acting as a foreign body, (2) the location of the fungus as to whether it is superficial or deep in the body, (3) endotoxins and the chemical constitution of the organism, (4) allergy and (5) chronicity of the process.

No one tissue change seems to be entirely characteristic of, or pathognomonic of, fungous disease.

INCLUSION DISEASE OF INFANCY

BERNARD KALFAYAN, M.D.
BEIRUT, LEBANON

THE FINDING of inclusion bodies in the tissues of infants has caused considerable interest and speculation. These bodies were most frequently found in the salivary glands and were identical morphologically with inclusions seen in the salivary glands of guinea pigs. Occasionally they have been found in more than one organ, and rarely were they spread throughout the body. The latter condition has been referred to as the "inclusion disease of infancy."

In 1926 Cole and Kuttner [1] succeeded in isolating a virus from the salivary glands of guinea pigs containing inclusion bodies. This virus is now known as the salivary gland virus of guinea pigs. However, no one has yet been able to demonstrate a virus in the salivary glands of infants similarly affected.

Von Glahn and Pappenheimer [2] in 1925, Farber and Wolbach [3] in 1932, Cowdry and Scott [4] in 1935 and Kinney [5] in 1942 reviewed the literature and added reports of cases of their own. Kinney stated that only 11 cases of the so-called inclusion disease of infancy had been reported, and he described a case of his own. I have been unable to find reports of new cases in the available literature after 1942. The present report is that of another case in which cellular inclusions were widespread in the viscera of an infant, with several observations hitherto undescribed.

REPORT OF A CASE

N. B., a 2½ month old baby, was admitted, Nov. 25, 1943, to the ophthalmology service of the American University Hospitals of Beirut with bilateral acute conjunctivitis, marked edema of the eyelids and high fever.

The parents had been married for six years and enjoyed excellent health. They had two other healthy living children, a boy of 6 and a girl of 4. There was no history of miscarriages, and the Wassermann and Kahn tests of the blood of both the parents and the children were negative.

The mother, a healthy multipara of 24 years, had an uncomplicated pregnancy and an easy delivery at home. The baby appeared normal at birth, took to

1. Cole, R., and Kuttner, A. G.: J. Exper. Med. **44**:855, 1926.
2. Von Glahn, W. C., and Pappenheimer, A. M.: Am. J. Path. **1**:445, 1925.
3. Farber, S., and Wolbach, S. B.: Am. J. Path. **8**:123, 1932.
4. Cowdry, E. V., and Scott, G. H.: Am. J. Path. **11**:647, 1935.
5. Kinney, T. D.: Am. J. Path. **18**:799, 1942.

breast well and started growing normally. When· he had reached the age of 1 month, conjunctivitis developed in the right eye, which persisted in spite of local treatment, and after a few weeks the inflammation passed to the left eye. Soon an exudate appeared on the right bulbar conjunctiva, like a membrane, and the possibility of diphtheritic conjunctivitis was considered. Six injections of antidiphtheritic serum were given, without improvement, and shortly afterward the eyelids became swollen, closing the eyes completely. The child began coughing, high fever developed and two to three days later the patient was admitted to the hospital.

On admission the positive findings were: bilateral conjunctivitis and edema of the eyelids, more on the right side; ground glass opacities of the right cornea; tracheobronchitis with possible consolidation of the lungs, although breathing was not unduly rapid or labored; spleen 3 cm. and liver 2 cm. below the costal arch; temperature 39 C. (102.2 F.); white blood cells, 22,700 per cubic millimeter, with 88 per cent polymorphonuclear neutrophils and 12 per cent lymphocytes. Culture of the conjunctival discharge of each eye revealed hemolytic streptococci. Culture of material swabbed from the throat showed hemolytic streptococci and Streptococcus viridans, Diplococcus pneumoniae and Micrococcus catarrhalis. Urinary findings (routine, microscopic and bacteriologic) were normal. Repeated blood cultures remained sterile. The Wassermann and Kahn tests of the blood were negative.

Local treatment (mild silver protein and boric acid) was instituted for the conditions of the eyes and sulfapyridine was given. On the second day the patient's temperature dropped to 38 C. (100.4 F.), but the following day it went up to 40 C. (104 F.) and a day later to 41 C. (105.8 F.), and the child became cyanosed and its abdomen distended. In the meanwhile, the conjunctivitis improved decidedly, and the respirations remained regular and easy. Sulfapyridine was substituted by sulfathiazole, 0.125 Gm. every four hours night and day, following which the temperature dropped abruptly from 41 to 36 C. (105.8 to 96.8 F.) within twenty-four hours. However, it then went up to 38 C. and remained at that level for two days, and thereafter it fluctuated between 37.5 and 38 C. (99.5 to 100.4 F.) for a week. During this period the child improved steadily, but the leukocyte count of about 16,000 per ·cubic millimeter and the fever could not be explained. Fluoroscopic and roentgenologic examinations of the chest showed clear lungs with moderately enlarged shadows at the hili. On the fifteenth and sixteenth days the temperature dropped further to 37 to 37.5 C. (98.6 to 99.5 F.), with corresponding clinical improvement. However, on the seventeenth day, for no apparent reason the child suddenly went into a state of shock, became cyanosed and remained in this state for· twelve hours and died. During this period he changed remarkably from a healthy-looking child to a state bordering on marasmus. It was also during this period that attention was first drawn to the enlarged submaxillary glands.

Autopsy (thirty-nine hours after death, the body having been preserved in the refrigerator at about 5 C.).—The body was that of an emaciated and cyanosed boy. The weight was 3,130 Gm.; the length, 4 cm. The right eye showed conjunctivitis and diffuse corneal opacities. The left eye appeared normal. Both submaxillary salivary glands were uniformly enlarged and congested. The thyroid gland was of normal size and shape but firm in consistency. The thymus was atrophic, particularly the right lobe. The trachea and bronchi were filled with hemorrhagic purulent exudate. The pleural sacs were normal. As to the lungs, both apexes appeared normal; the rest showed patchy consolidation with areas

of firm consistency. The tracheobronchial lymph nodes were enlarged and congested. The pericardial sac, the heart and the great vessels appeared normal. The peritoneal sac appeared normal. The stomach and intestines were distended with gas. The liver was uniformly and moderately enlarged and firm; its external surface was smooth, and the cut surface had a mottled appearance. The gallbladder and the biliary passages were normal. The spleen was uniformly and moderately enlarged and firm; its surface was smooth. The adrenal glands and the kidneys were congested. The pancreas and the testes appeared normal.

Tissues were fixed in a 4 per cent solution of formaldehyde and in Zenker's solution to which solution of formaldehyde U.S.P. was added to a concentration of 5 per cent, and were stained with hematoxylin-eosin.

The conjunctiva of the right eyeball was destroyed. The cornea, the palpebral conjunctiva and the sclerocorneal junction were infiltrated by numerous polymorphonuclear neutrophils, eosinophils, large monocytes (many actively phagocytic) and lymphocytes. From the sclerocorneal junction the inflammation could be traced outward into the episcleral tissues and the extrinsic muscles of the eye, where the lesions were of a granulomatous nature and located mainly around blood vessels, and inward to the ciliary body and the iris. There was a purulent exudate in the posterior chamber. The retina, the optic nerve and the lens were normal.

Several hypertrophied cells with acidophilic nuclear inclusions (fig. 1) were seen in the sclerocorneal junction, the episcleral tissues, the ciliary body and in areas of a more chronic inflammatory reaction. There were no cytoplasmic inclusions in these cells. (The description of the inclusions and the inclusion-laden cells is given under "Comment.")

The submaxillary glands were congested. Many epithelial cells lining the acini and the ducts were markedly hypertrophied, projecting into the lumen, and contained nuclear inclusions; some of these cells also contained cytoplasmic inclusions (fig. 2). The inclusion-laden cells could also be found free in the lumens of acini and ducts. Mild periductal and periacinic fibrosis and lymphocytic infiltration were seen where inclusions were present. Several ducts were distended with fluid, epithelial cells and lymphocytes.

The thyroid gland showed marked fibrosis throughout, with atrophic and empty acini. Occasionally hypertrophied acinic epithelial cells were seen containing nuclear and cytoplasmic inclusions.

The thymus was atrophic.

The lungs showed extensive and varied pathologic changes. There was widespread bronchopneumonia with numerous streptococci and pneumococci, alternating with areas of atelectasis. Here and there areas of fibrosis were seen with glandlike alveoli lined by cuboidal cells and with foci of small round cell infiltration. In these areas there were numerous markedly hypertrophied cells with nuclear inclusions, and some of these cells also contained cytoplasmic inclusions (fig. 3). These cells were found either among the alveolar lining cells or free in the alveoli (fig. 4). There was also extensive lipid pneumonia with numerous lipophages in the alveoli, particularly well seen in the atelectatic and fibrotic areas (fig. 5).

The tracheobronchial lymph nodes showed acute lymphadenitis.

Throughout the liver numerous small scars appeared, frequently located around the central veins. Close to the scars and sometimes merging with them were foci of large mononuclear leukocytes with occasional granulocytes. There was
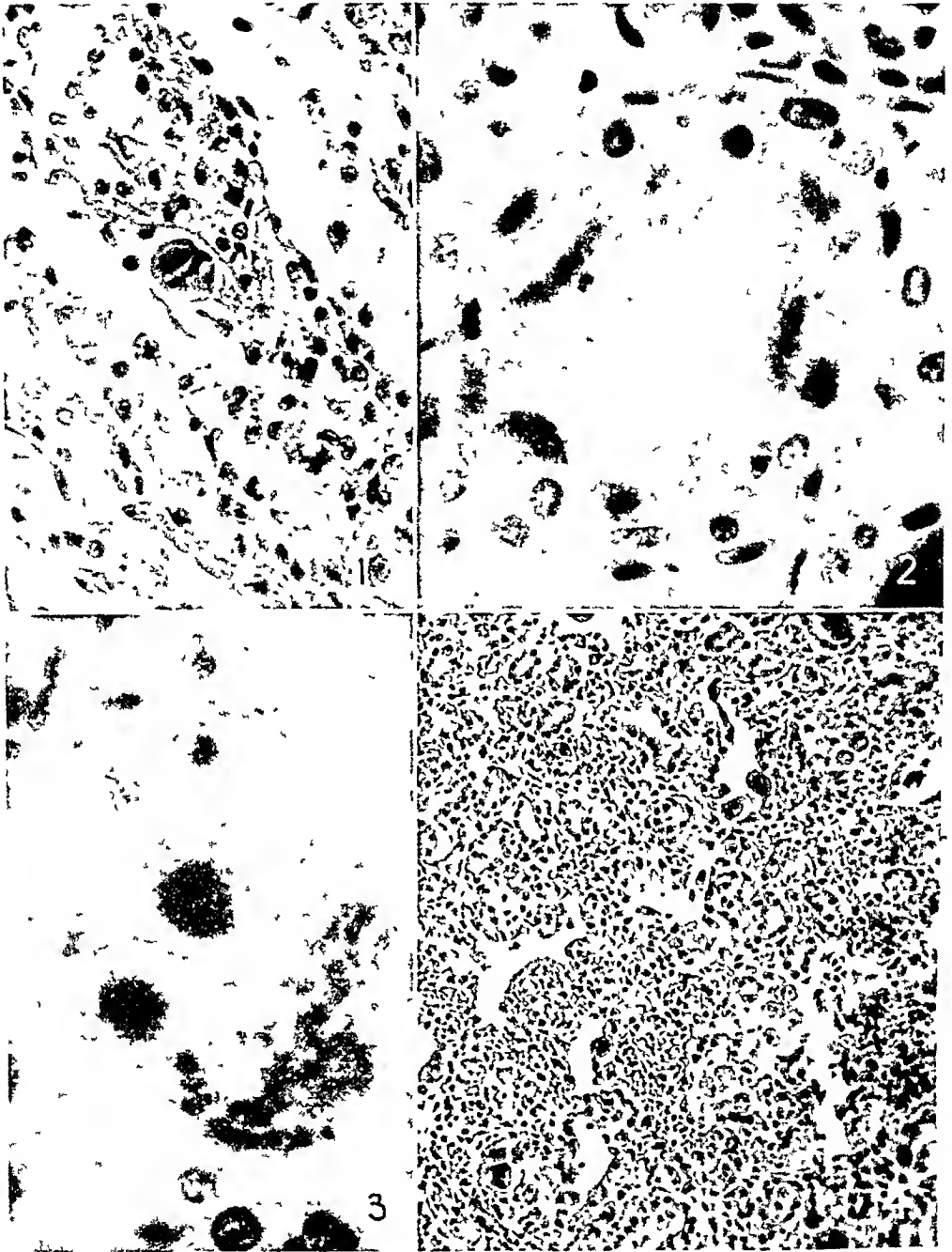
The stated magnifications are approximate.

Fig. 1.—Sclerocorneal junction of the eyeball. Note the hypertrophied cell with a nuclear inclusion and a cytoplasmic vacuole. × 375.

Fig. 2.—Small duct of the submaxillary gland, lined by hypertrophied cells with nuclear and cytoplasmic inclusions. × 850.

Fig. 3.—Hypertrophied binuclear alveolar lining cell of the lung. Each nucleus contains one inclusion; the cytoplasm contains numerous inclusions. Note the clear halo around one cytoplasmic inclusion. × 1,200.

Fig. 4.—Pulmonary area of chronic inflammation and fibrosis with gland-like alveoli. Note numerous hypertrophied cells projecting into or free in the lumens of alveoli and containing nuclear inclusions × 170
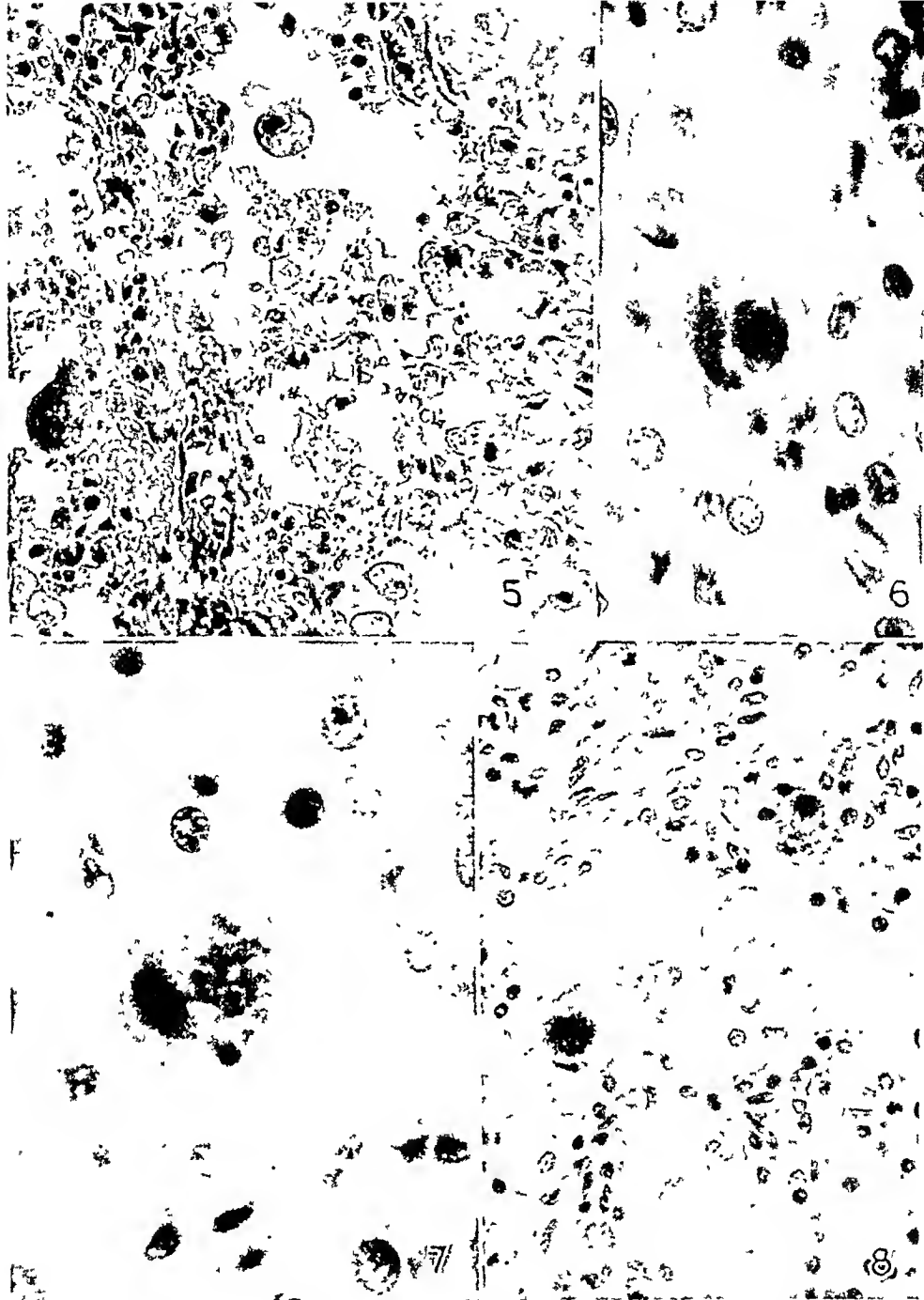
Fig. 5.—Lipid pneumonia. The alveoli contain numerous macrophages with vacuolated moth-eaten cytoplasm—lipophages. There is one hypertrophied cell with a nuclear inclusion and a well defined clear area (a vacuole) in the cytoplasm. The racket-shaped hypertrophied cell is filled with cytoplasmic inclusions but has no nucleus—a "pseudocyst" (see discussion of cytoplasmic inclusions under "comment," page 473). × 350.

Fig. 6.—A hypertrophied cell with both nuclear and cytoplasmic inclusions in the center of a collecting tubule of the kidney. × 950.

Fig. 7.—A hypertrophied cell with both nuclear and cytoplasmic inclusions in an otherwise normal islet of Langerhans. × 1,200.

Fig. 8.—In this section of lung a hypertrophied cell on the right contains a compact aggregate of cytoplasmic inclusions but no nucleus, simulating the pseudocyst of Toxoplasma. Note another hypertrophied cell with nuclear and cytoplasmic inclusions, and numerous lipophages. × 380.

The stated magnifications are approximate.

471

patchy congestion. In ten sections representing different parts of the liver only one hypertrophied cell with a nuclear inclusion was found among the epithelial cells of a small bile duct.

The spleen was congested and showed mild fibrosis throughout. In five sections examined two hypertrophied cells with nuclear inclusions were seen.

The adrenal glands contained several large areas of coagulation necrosis near the central vein. In one such area numerous hypertrophied cells with nuclear inclusions were seen; here the central vein was thrombosed. However, similar cells were also rarely seen in the cortex with no evidence of inflammation around them. Here and there foci of small round cell infiltration were observed.

The kidneys were congested. A few glomeruli were fibrotic. Occasional hyaline casts were seen in the collecting tubules. In the lumen of one otherwise normal collecting tubule, there was a free hypertrophied cell containing both nuclear and cytoplasmic inclusions (fig. 6).

The pancreas did not show any lesions. However, in one otherwise normal islet of Langerhans there was a hypertrophied cell containing both nuclear and cytoplasmic inclusions (fig. 7).

The testes showed no lesions.

Levaditi's silver impregnation revealed no spirochetes on repeated examination of several sections of the liver, the lungs, the adrenal glands and the pancreas.

In bacteriologic examinations, hemolytic streptococci and Diplococcus pneumoniae were cultured from both lungs. Cultures of heart blood, liver, spleen and kidneys remained sterile.

SEARCH FOR VIRUS

The nature of the case not being suspected clinically or at autopsy, no attempts were made to demonstrate a virus in the tissues. However, when the mother had a subsequent delivery in the hospital, the placenta obtained was used for transmission experiments with the possibility in mind that there might be a congenital virus infection with the mother acting as carrier. The chorioallantoic membranes and the yolk sacs of developing chicken embryos, guinea pigs and white mice were inoculated with a saline emulsion of sterile pieces of placenta. The results were entirely negative.

COMMENT

The Hypertrophied Cells and the Inclusions (table)—The hypertrophy, affecting about equally the nucleus and the cytoplasm, was sufficiently definite to make detection easy under low magnifications. The average enlargement of the cell was about two to four times the normal, but on the whole it was more marked where cytoplasmic inclusions were also present.

The nuclear inclusions were two to three times as large as the unaffected nuclei. They were round or oval, homogeneous, acidophilic bodies that were separated from the nuclear membrane by a clear halo. The remains of the nuclear chromatin were seen as coarse basophilic particles on or near the nuclear membrane. Variations from these characteristic inclusions were seen now and then. A tiny red droplet or a red streaklike structure in an enlarging nucleus was considered as

an early stage in the development of an inclusion, whereas disappearance of the clear halo, the central inclusion merging with the nuclear membrane and becoming more basophilic, was taken as evidence of degeneration of the inclusion.

In a given nucleus only one inclusion was present. Multinuclear hypertrophied cells were seen most commonly in the lungs (figs. 3 and 4) and less frequently in the salivary glands and the thyroid gland. Each nucleus in such a cell contained one inclusion. In one such cell in the lung eight nuclei were counted, each with one inclusion. The multinuclear cells were formed by fusion of continuous cells, as partial fusion of adjacent cell walls could clearly be seen in several instances.

The cytoplasmic inclusions were multiple spherical bodies, faintly basophilic, located along the cell wall opposite the nuclear pole. Although fairly uniform in size and shape in a given cell, they varied from fine powdery material (virus aggregate?) to typical globular inclusions in

Distribution of Inclusion Bodies

Organ	Hypertrophied Cells with Inclusions				Inclusions		Inflammatory Reaction		
	Number			Multi-nuclear Type	Nuclear	Cyto-plasmic	Marked	Slight	Absent
	Numerous	Occasional	Very Few						
Eyeball......................	..	+	..	−	+	−	+	..	..
Submaxillary salivary gland	+	..	..	+	+	+	..	+	..
Thyroid gland...............	..	+	..	+	+	+	..	+	..
Lungs.......................	+	..	..	+	+	+	+	..	..
Liver.......................	..	..	+	−	+	−	+	..	..
Spleen......................	..	..	+	−	+	−	..	+	..
Adrenal glands..............	+	..	..	−	+	−	+	..	..
Kidneys.....................	..	..	+	−	+	+	..	+	..
Pancreas....................	..	..	+	−	+	+	..	..	+

different cells. Not infrequently the cytoplasm around the inclusions was vacuolated, and occasionally "moth-eaten" cytoplasm was seen without any inclusions.

Frozen sections of the lungs stained for fat revealed, besides numerous lipophages, fat droplets in a few of the inclusion-laden hypertrophied cells, some of which also contained blood pigment. It was hard to determine whether these cytoplasmic contents were acquired by phagocytic activity or resulted from cellular degeneration. Scarlet red and sudan III stains did not show any difference in the staining of the lipid droplets in the lipophages and in the inclusion-laden cells. However, osmic acid was not precipitated by the fat in the lipophages but was precipitated by the fat in the hypertrophied cells.

Cytoplasmic inclusions were present in cells containing nuclear inclusions and were not found in cells with normal nuclei. Occasionally in the lungs and the salivary glands a hypertrophied cell was seen filled

with cytoplasmic inclusions but with no visible nucleus (figs. 5 and 8), closely resembling the so-called pseudocyst of Toxoplasma as described by Sabin and others,[6] from which they could be differentiated, however, morphologically and by staining characteristics. These cystlike cells probably were formed by oblique sectioning of the cytoplasmic inclusion pole of the cell or by the degeneration or the extrusion of the nucleus as suggested for the pseudocysts of Toxoplasma.

The cytoplasmic inclusions were found only in the epithelial cells, i. e., cells lining alveoli, acini, ducts or tubules (submaxillary gland, thyroid gland, lungs, kidneys and pancreas); they were not found in the macrophages in areas of inflammation or in nonepithelial organs, where nuclear inclusions alone were found (eye, spleen and adrenal glands).

The Significance of Inclusions.—The most plausible and probable cause of inclusions, namely, viruses, has been contested in these cases mainly on the ground that no one has yet succeeded in isolating a virus from infant tissues containing inclusions. Moreover, the finding of inclusion bodies in the tissues—particularly the salivary glands—of syphilitic infants and in those who have died· with clinical whooping cough, as well as the fact that inclusions have been experimentally produced by the use of nonliving agents, has put the virus theory on a still less firm ground.

The earliest reports (quoted by Farber and Wolbach) on inclusion bodies in the tissues of infants mention not infrequently the coexistence ·of congenital syphilis. In the present case syphilis was strongly suspected on the basis of histologic changes observed in sections of liver, lungs and eyeball, namely foci of fibrosis and chronic inflammation. However, syphilis was ruled out because of: (1) negative Wassermann and Kahn tests of the blood; (2) repeated failure to find spirochetes in silver-impregnated sections of liver, pancreas, adrenal gland and lung; (3) negative clinical and serologic findings in both parents and children (4) the maternal history of three normal pregnancies and no miscarriages, with a fourth normal pregnancy and delivery of a healthy baby a year after the death of the child under discussion. It is quite probable that in some of the early reported cases syphilis was diagnosed on histologic observations alone. De Lange[7] made the more cautious diagnosis of "probably syphilitic" from histologic observations alone in a case in which the Wassermann and Kahn tests were negative and spirochetes were not found in the sections. All of this seems to indicate that the inclusions and the inflammatory changes are etiologically related.

6. Sabin, A. B.: Recent Advances in Pediatrics, London, William Heinemann, 1942, vol. 1, pp. 1-56.

7. de Lange, C.: Virchows Arch. f. path. Anat. **237:**276, 1922.

Rich[8] and McCordock[9] were among the first to report the finding of intranuclear inclusions associated with whooping cough. Kinney reported finding intranuclear inclusions in the lungs at 4 and in the salivary glands at 2 autopsies of infants suffering from whooping cough; cytoplasmic inclusions were present only in the salivary glands. In a series of 35 cases of pertussis cited by Kinney, intranuclear inclusions were found in the lungs or the parotid glands in 16 per cent. The significance of inclusions observed in the lungs and the salivary glands in cases of pertussis remains undetermined. Furthermore, there is no clinical or bacteriologic evidence that the child under discussion was suffering from whooping cough.

Several investigators have succeeded in producing inclusions in animals through the use of nonliving agents. Olitzky and Harford[10] claimed to have produced intranuclear inclusions morphologically indistinguishable from the amorphous acidophilic virus inclusions by injecting aluminum oxide into guinea pigs. Their work was later confirmed by Birch and Lucas.[11] However, judged from the photomicrographs, the inclusions they produced differ from the salivary gland inclusions of guinea pigs and from those observed in the present case in several important aspects. The nuclear and cellular hypertrophy are not so marked, the nuclear inclusion are not so large and uniform, and cytoplasmic inclusions are absent in their cases. In the present case the child apparently had not received any chemical substance that could be accused of having produced inclusions, according to present day knowledge.

The morphologic similarity between the salivary gland inclusions of guinea pigs and those of infants has naturally led to the conclusion that the latter were also produced by a virus. McCordock and Smith[12] and Kuttner and Wang[13] failed to obtain a virus from infant tissues containing inclusions. Adams[14] reported similarly that no virus could be recovered from the lungs of infants suffering from so-called "primary virus pneumonia" though cytoplasmic inclusions were seen in the bronchial epithelial cells. These failures cannot be taken as evidence against the virus theory, because of adverse experimental conditions resulting from lack of clinical signs suggesting the nature of the pathologic condition. The finding by Farber and Wolbach of inclusions in

8. Rich, A.: Bull. Johns Hopkins Hosp. **51**:346, 1932.

9. McCordock, H. A.: Proc. Soc. Exper. Biol. & Med. **29**:1288, 1932.

10. Olitzky, P. K., and Harford, C. G.: Am. J. Path. **13**:729, 1937; Proc. Soc. Exper. Biol. & Med. **38**:92, 1938.

11. Birch, F., and Lucas, A.: Am. J. Path. **18**:1051, 1942.

12. McCordock, H. A., and Smith, M. G.: Am. J. Dis. Child. **47**:771, 1934.

13. Kuttner, A., and Wang, S. H.: J. Exper. Med. **60**:773, 1934.

14. Adams, J. M.: J.A.M.A. **116**:925, 1941.

the salivary glands of 12 per cent of infants in their series of 183 autopsies leaves little doubt that the condition is far from being rare. If it is caused by a virus, the chances of recovering the virus would probably be great if routine transmission experiments were carried out on such large series.

SUMMARY

A rare case of "inclusion disease of infancy" is described, with the clinical and postmortem findings. The significance of inclusion bodies is considered.

The inclusions were found in the submaxillary salivary glands, the lungs, the thyroid gland, the eye, the adrenal glands, the spleen, the pancreas, the kidneys and the liver.

The findings in the case were (*a*) lesions of the eye, the liver and the adrenal glands and (*b*) the presence of hypertrophied cells with no visible nuclei but with multiple cytoplasmic inclusions, simulating the "pseudocysts" of Toxoplasma.

PRIMARY MELANOBLASTOMA OF THE CEREBELLAR LEPTOMENINGES WITH WIDESPREAD EXTRACRANIAL METASTASES

Report of a Case

RICHARD M. STRONG, M.D.

KANSAS CITY, MO.

ALTHOUGH nearly a century has elapsed since the first invasive melanoma of the meninges was reported, this tumor still is held to be a rarity in oncology. In 1935 Akelaïtis[1] published a comprehensive review of the literature and an additional case. Since that time there have been only sporadic reports of cases of primary meningeal invasive melanoma. Schnitker and Ayer[2] abstracted reports of 30 cases and added a case of their own in 1938. In the two succeeding years, 4 cases were reported, 2 by Jütte[3] and 2 by Ray and Foot.[4] Since 1940 there have been three reports, those of Christensen,[5] Mackay and Hurteau[6] and Neubuerger, Daniels and Draper.[7] In the last two reports, the tumors were not thought to have their primary origin in the leptomeninges. A review of the reports made since 1935, therefore, reveals a total of 36 cases of cancerous melanotic tumor taking origin from the leptomeninges. In these cases the tumor showed either invasion or metastasis or both. In the majority of these cases no metastatic deposits were found outside the central nervous system, the exceptions being the cases reported by Boit[8] (liver and spleen), Ehnmark and Jacobowsky[9] (liver) and Foot and Zeek[10] (lungs—2 cases).

It is the purpose of this paper to present a case of malignant melanoblastoma of the cerebellar meninges in which there were amelanotic

From the Department of Pathology, Veterans Administration Hospital.

1. Akelaitis, A. J. E.: Am. J. Path. 11:591, 1935.

2. Schnitker, M. T., and Ayer, D.: J. Nerv. & Ment. Dis. 87:45, 1938.

3. Jütte, H.: Virchows Arch. f. path. Anat. 304:296, 1939.

4. Ray, B. S., and Foot, N. C.: Arch. Neurol. & Psychiat. 44:104, 1940.

5. Christensen, E.: Acta chir. Scandinav. 85:90, 1941.

6. Mackay, F. H., and Hurteau, E. F.: J. Nerv. & Ment. Dis. 96:369, 1942.

7. Neuberger, K. T.; Daniels, L. E., and Draper, P. A.: J. Neuropath. & Exper. Neurol. 2:140, 1943.

8. Boit, H.: Frankfurt. Ztschr. f. Path. 1:248, 1907.

9. Ehnmark, E., and Jacobowsky, B.: Upsala lakaref. förh. 31:565, 1926.

10. Foot, N. C., and Zeek, P.: Am. J. Path. 7:605, 1931.

metastases in the skin, lymph nodes, the left lung, the right adrenal gland and the stomach.

REPORT OF CASE

A 49 year old white man first entered Wadsworth Veterans Hospital on April 4, 1946, complaining of abdominal distention. The past history of the patient and that of the family were noncontributory. The illness began March 25, 1946 with abdominal cramps, nausea and vomiting. These symptoms persisted for two days, opiates being administered for relief of pain. He was finally taken to Independence Sanitarium and Hospital, Independence, Mo., where he was operated on by Dr. Raymond Gard. On communicating directly with Dr. Gard I learned that an inflamed Meckel's diverticulum had been removed and a volvulus around a fibrous band in the right lower quadrant of the abdomen released, that gross and micro-scopic examination of the diverticulum had shown nothing but acute inflammation, that the postoperative course.had been uneventful, and that the patient had been discharged.

When the patient was examined on entering this hospital, the positive findings were limited to the abdomen, which was distended, showed a well healed midline scar and a reducible right inguinal hernia. The red blood cell count was 4,740,000, and the hemoglobin content was 91 per cent. Other laboratory findings were nega-tive, as were the results of roentgen examination. Miller-Abbott decompression was instituted as well as parenteral administration of fluids. The patient responded well and was discharged on April 16, 1946.

He reentered the Wadsworth Veterans Hospital on October 3, complaining of continuous pain of the left side of the chest. During the interval he had noticed no symptoms until three months prior to admission, when he fell from a horse and injured the left side of his chest. The chest was taped for some time, but he· experienced no relief. He stated that although he had gained weight since his previous admission, he had lost 15 to 20 pounds (6.5 to 9 Kg.) in the past three months.

The patient was a well developed, undernourished white man, 5 feet 8 inches in height (172.5 cm.) and weighing 136 pounds (61.5 Kg.). His temperature was 100.2 F., pulse rate, 110; blood pressure, 110 systolic and 70 diastolic; respirations, 20. He presented a picture of recent loss of weight and a somewhat cachectic appearance. There was complete flatness to percussion over the left side of the chest with absence of breath sounds and absence of vocal and tactile fremitus. A roentgenogram of the chest showed increased density in the upper lobe of the left lung and a pleural effusion with a fluid level at the fourth rib anteriorly. A barium sulfate meal and a barium sulfate enema revealed a marked narrowing of the pelvic bend of the colon, but otherwise nothing remarkable. The red cell count was 2,870,000; the hemoglobin content, 56 per cent; the white cell count was 17,400, with a shift to the left; the sedimentation rate was 15 mm. per hour (Westergren).

On October 5, thoracentesis of the left side was done, and 1,250 cc. of blood-tinged fluid was removed. No tumor cells were found in the stained sediment. Daily blood counts showed increasing anemia and a gradual fall of the white cell count. On October 14, a nontender small hard nodule occurring above the left clavicle was discovered, and a small hard mass, measuring 2 by 3 cm. was encountered to the left of the umbilicus just lateral to the old midline scar. Neither of these had been noticed on entry, and the patient had been aware of them for only two days prior to their discovery on October 14. October 15, a biopsy specimen was taken from each of these areas.

The microscopic examination of the mass in the neck revealed a highly cellular and partially necrotic tumor replacing a lymph node. The markedly anaplastic cells were arranged in sheets and bands, separated by thin strands of fibrous septums. They were polyhedral, with pale pink-staining cytoplasm and bizarre-shaped nuclei showing both hyperchromatism and hypochromatism (fig. 1). Many mitotic figures were encountered. The specimen taken from the abdominal wall showed essentially the same picture.
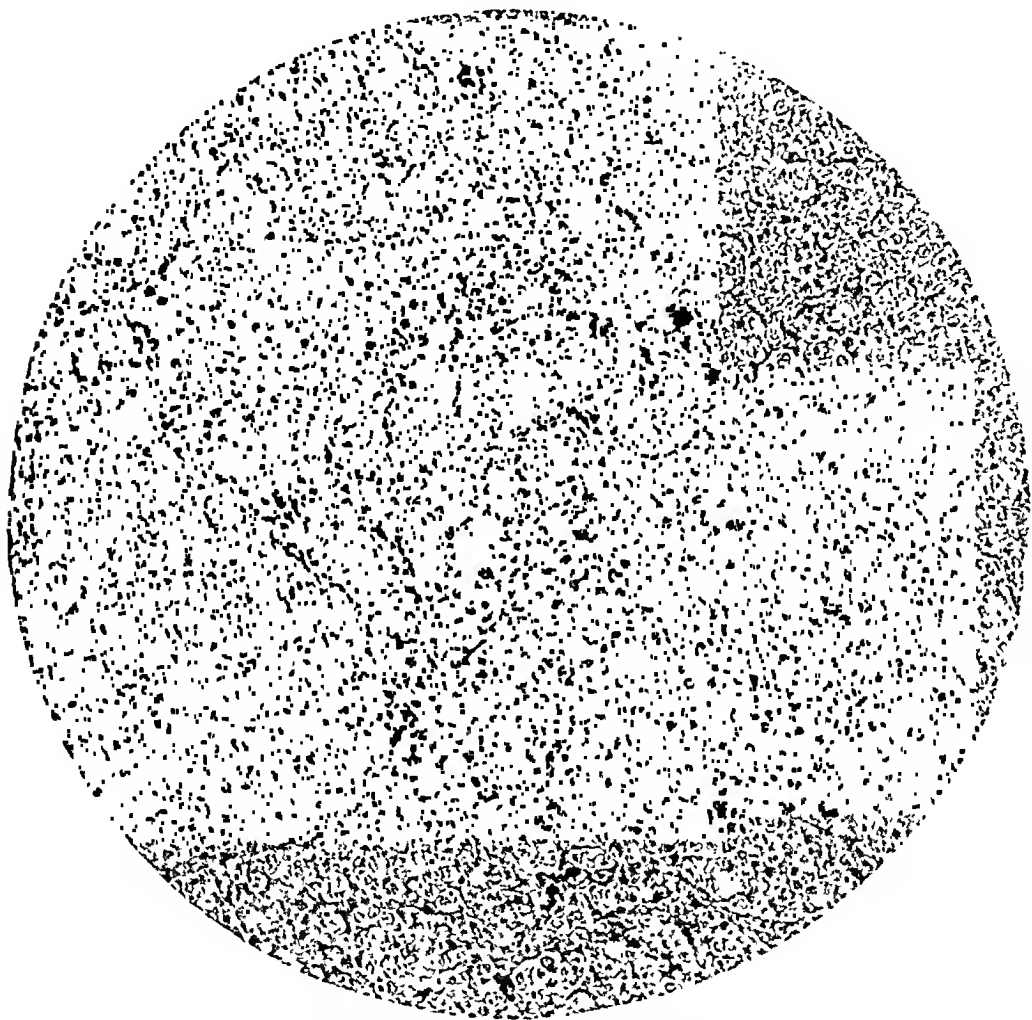
Fig. 1.—Low power photomicrograph (× 135) showing melanoblastoma in a cervical lymph node. Note diffuse growth of anaplastic tumor cells largely replacing the gland.

The patient grew progressively worse, and even repeated blood transfusions failed to raise the red blood cell count above 1,500,000. November 4, he began vomiting large amounts of blood, and he died on November 5.

Necropsy (four hours after death).—Other than passive congestion of the viscera, the changes observed were of a neoplastic nature. On examination of the surface of the body, hard, matted nodes were palpated in the left cervical region and both inguinal regions. Just beneath the skin to the left of the umbilicus was a

firm mass, measuring 6 by 6 cm. Cut section showed it to be highly cellular in appearance, with color ranging from white to light brown.

The left lung was the site of a metastatic tumor incorporating the upper lobe and measuring 6 by 9 cm. This tumor extended partially around the trachea and the ascending aorta and involved the left hilar lymph nodes.
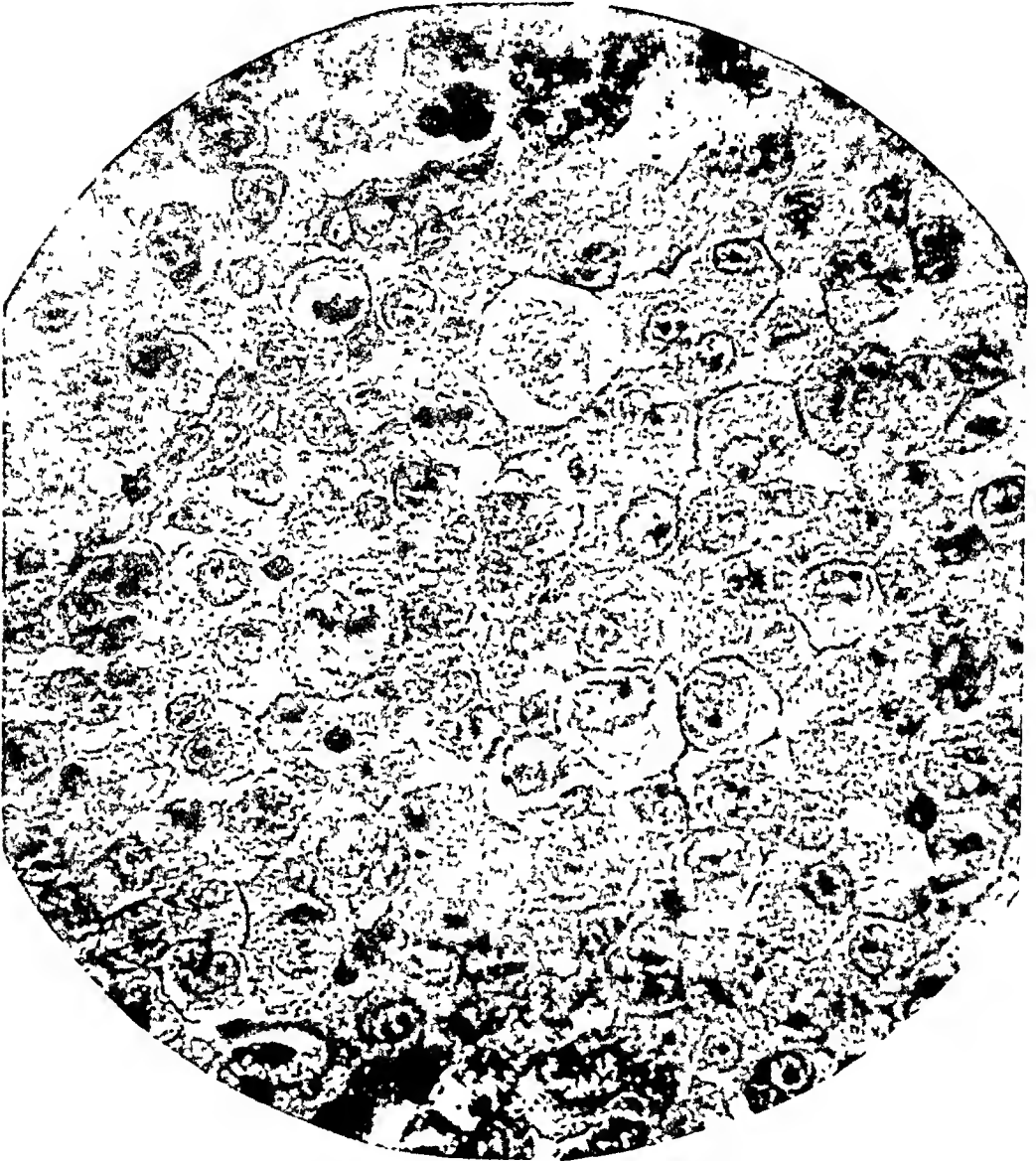
Fig. 2.—High power photomicrograph ($\times$ 600) of cerebellar melanoma showing a solid mass of tumor cells. Note the marked nuclear changes and mitotic figures.

The right adrenal gland showed its superior portion replaced by a firm tumor, 2 cm. in diameter.

The abdominal cavity revealed widespread tumor invasion of the mesenteric and retroperitoneal lymph nodes, these being both matted and discrete. All these

metastases were whitish gray and firm in consistency; many showed areas of softening and hemorrhage. The stomach had an ulcerous tumor deposit, 3 cm. in diameter, on the lesser curvature 4 cm. from the pylorus. Two smaller nodules were invading the gastric wall. These were discrete pinkish red tumors, showing no deposition of pigment.

The cerebral parenchyma and meninges were not remarkable. However, serial sections of the cerebellum brought out a small brownish black area adjacent to the vermis on the left side. This occupied anatomically a part of the biventral lobe and was irregular in outline, measuring 1 by 1 by 1.5 cm. The mass was soft, and its resemblance to an area of hemorrhage could not be dismissed. Although it was strongly suspected of being the primary tumor, proof of this area's malignant properties was not obtained until microscopic sections were examined.

Microscopic Examination.—Histologic changes other than those referable to the tumor and its metastases included mild prostatic hypertrophy, mild passive congestion of the liver and the spleen, and cloudy swelling of the kidneys. Portions of the lungs not invaded by tumor revealed pulmonary edema and small areas of atelectasis.

The tumor, in all the invaded organs, as well as in the cerebellum, presented a picture of a highly cellular growth with scanty stroma and large areas of necrosis. The cells, for the most part, were arranged in fairly well circumscribed groups or sheets, surrounded by a thin border of strandlike, spindle-formed stroma cells· (fig. 2). No arrangement suggesting direct association with blood vessels could be made out, but in all the areas invaded by tumor the vessel walls were surrounded by tumor cells. Several nests of anaplastic cells were encountered in the lumens of vessels.

The cells were likened to those of one type of tumor described by Ewing,[11] being large round or polyhedral cells with relatively pale cytoplasm. Many bizarre-shaped nuclei were encountered, some showing hyperchromatism and some hypochromatism. Mitotic figures were numerous. The fibrous-like stromal elements were relatively sparse, being crowded out by the remarkable cellularity of the growth.

In the cerebellum the tumor was rather well demarcated, being bound by the meningeal folds of the cerebellar sulci. Necrosis of the tumor was evident in several areas, these showing strands of fibrin, blood and anuclear cellular remnants. Definite deposition of brownish black pigment was evident in the tumor cells adjacent to the meninges. Iron stains revealed part of this pigment to be hemosiderin, but many granules located in the tumor cells were untouched by the deposition of iron and retained the black color. Silver stains done according to Foot's modification of Bielschowsky's method showed definite fibrils connecting the tumor cells with the adjacent leptomeninges (fig. 3). These fibrils had the same appearance as those described by Foot and Zeek, arborizing about the tumor cells in a dense network. Furthermore, the granules within the tumor cells stained a deep black, giving additional support to the belief that melanin was present. Iron stains were done on the metastatic lesions, and section of a perirenal node revealed a few scattered cells containing coarse black granules which did not take the blue color of the iron stain. In no area did the stroma show deposition of pigment such as is frequently noted in melanotic tumors.

The stomach showed tumor cell invasion throughout all layers, these being destroyed and infiltrated by large, markedly atypical cells.

---

11. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 955.

vividly the deep black color of these granules, this giving further support to the claim that melanin was present. The sections of metastatic deposits showed no melanin with the exception of the one metastasis in a perirenal node, but the cytologic characteristics were those of a malignant amelanotic melanoblastoma. There was no diffuse melanomatosis of the meninges; this has not infrequently been a characteristic finding.

The widespread metastases reported here were of interest not only because of their varied dissemination but also because of the small, asymptomatic primary lesion.

All other possible primary sites were carefully examined, but the presence of black pigment in the tumor cells, the location of the lesion, its gross appearance and the staining properties noted with iron and silver impregnations led to the belief that the cerebellar neoplasm was the primary tumor.

Case Reports

CYSTIC MEDIONECROSIS OF THE AORTA

D. MAGNER, M.D., and H. F. McKENZIE, M.D.

REGINA, SASKATCHEWAN, CANADA

CYSTIC degenerative changes occurring in the aortic medial coat have been recognized for many years. Erdheim's [1] name is usually associated with the condition, which he termed "medionecrosis aortae idiopathica cystica." It has been noted that these changes occur mainly in old age and are generally associated with aneurysm of the dissecting type. The case presented here is unusual in that the condition occurred in a young man and resulted in a large fusiform aneurysm of the ascending portion and arch of the aorta. Death was due to massive intrapericardial hemorrhage originating from a small perforation in the wall of the aneurysm.

REPORT OF CASE

A 20 year old white man of Anglo-Saxon stock was admitted to the Regina Cancer Clinic, July 6, 1943, from the Royal Canadian Air Force. The routine enlistment roentgen examination had revealed, June 10, 1943, "a solid upper mediastinal tumor, possibly a thymoma, surrounding the great vessels."

The patient stated that he had had no previous illness and had always been in excellent health. He could not recall having had any of the usual infectious diseases of childhood. He recollected that in January or February 1941, immediately after his participation in an ice hockey game, a severe pain developed in the upper part of the chest behind the "breast bone." The pain occurred in spasms, apparently related to changes in position. He remained in bed for three days at that time, but did not consult a physician. After three days the pain vanished and did not reappear. In January 1943 a cold developed, accompanied by a deep-seated cough, unproductive of sputum. This, he stated, had persisted in a mild form. Otherwise, he felt perfectly well.

Physical examination gave entirely negative results except for the findings in the chest. The cardiac apical impulse was forcible in the fifth intercostal space and behind the sixth rib, approximately 7 cm. from the midline of the sternum. Percussion revealed the right border of the heart to be 4 cm. from the midline, in the third and fourth intercostal spaces. The heart sounds were loud, and there was an apical systolic murmur. Over the right cardiac area there was a moderately rough systolic murmur, maximal over the sternum at the level of the third interspace. The pulmonary second sound was accentuated. A transmitted systolic murmur was audible in the interscapular area. Breath sounds were increased over the right side of the chest, but no adventitious pulmonary sounds were heard.

1. Erdheim, J.: Virchows Arch. f. path. Anat. 276:187, 1930.

The blood pressure was not recorded but is stated to have been normal. An electrocardiogram was reported as consistent with slight enlargement of the right side of the heart. The erythrocyte count was 4,600,000 per cubic millimeter and the hemoglobin content 15.6 Gm. per hundred cubic centimeters of blood; the *leukocyte count was 8,950 per cubic millimeter, of which 51 per cent were neutro-phils, 37 per cent lymphocytes and 5 per cent disintegrated cells.* The erythrocytes revealed no morphologic abnormality. The urine showed a slight trace of albumin but was otherwise normal. The Kahn test of the blood was negative. Roentgen examination of the chest revealed a large mass in the upper part of the mediastinum,
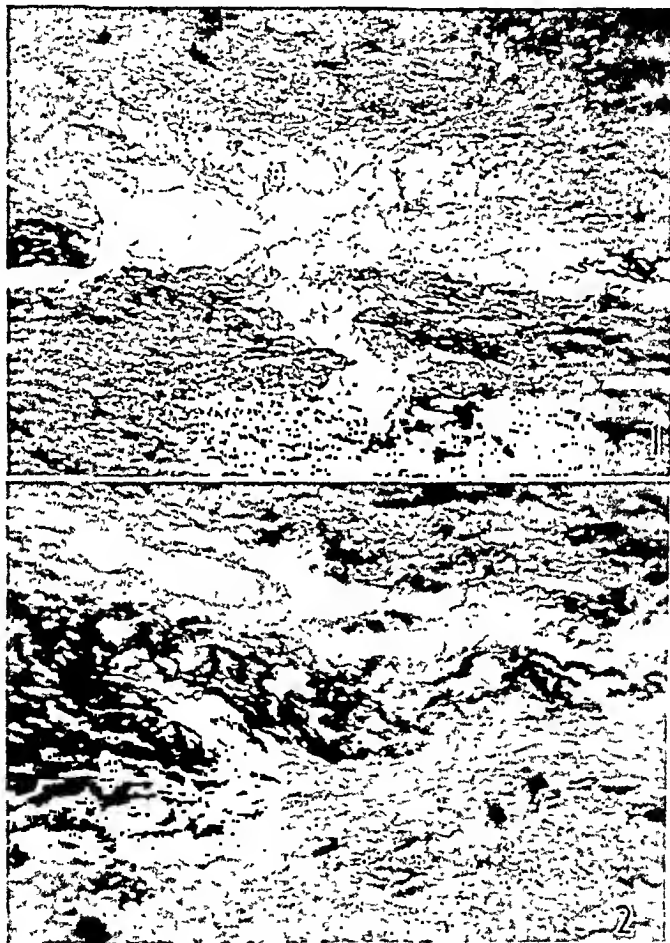
Fig.1.—Wall of aortic aneurysm. A cystic accumulation of mucoid material is seen, and there is a disappearance of muscle and elastic fibers. Verhoeff's stain for elastic tissue; × 100.

Fig. 2.—Wall of aortic aneurysm. The remaining elastic fibers are swollen and fragmented. An accumulation of mucoid material is seen. Verhoeff's stain for elastic tissue; × 450.

which on fluoroscopic examination showed pulsation. The radiologist believed that this represented an aneurysm of the ascending aorta.

The patient returned to civilian work as a clerk in a hardware store and carried on in an entirely satisfactory manner. There were no respiratory or

cardiovascular symptoms, and he maintained his weight and strength. He was reexamined frequently but at irregular intervals. In January 1945 a roentgen examination showed a definite increase in the size of the upper mediastinal mass. After that, he was not seen until Jan. 11, 1946. He still felt well and was free of symptoms save that on that day he had experienced a momentary severe shooting retrosternal pain, which had not recurred. Roentgenograms of the chest, however, showed still further increase in the transverse diameter of the mass, mainly to the right. On February 1 bronchoscopic examination revealed what seemed to be external pressure on the trachea in the lower third, with some distortion of the left main bronchus. There was noticeable pulsation in the trachea. Five days later, on the evening of February 6, the patient complained of excessive tiredness and mild dizziness. He lay down to rest and within ten minutes was discovered to be comatose, cyanotic, cold and sweating. Death occurred shortly afterward as he was on the way to the hospital in an ambulance.

Necropsy (twelve hours after death).—The pericardial sac contained 700 cc. of dark red jelly-like blood clot. There was a large fusiform aneurysm of the ascending portion and the arch of the aorta. The dilatation commenced immediately distal to the aortic valve cusps and extended to just beyond the origin of the left subclavian artery. From this point on, the aorta was of a normal diameter of 5 cm. The aneurysm measured 10 5 cm. in length and was 15 cm. in diameter at its widest portion. It was semicollapsed. A small break, 0.5 cm. in length, situated in the anterior wall just distal to the aortic orifice, was evidently the source of the massive intrapericardial hemorrhage. The remainder of the intimal surface of the aneurysm was smooth and creamy white except for an oval area 4 cm. in long diameter, situated on the posterior wall 2.5 cm. distal to the aortic orifice. This area was slightly depressed and showed an intima coarsely corrugated by orange-yellow atheromatous streaks. Plaques of calcium were present in the wall in this region. Elsewhere the aneurysmal wall was uniformly and diffusely thickened by dense grayish white fibrous tissue in the adventitia, but no other zones of calcification were present. The aorta below the aneurysm was taut and elastic, and the intima showed a minimal amount of fatty streaking. The great vessels of the neck and the iliac vessels were not remarkable.

The heart weighed 325 Gm. It was covered by smooth, glistening pericardium. The chambers were of normal size and had smooth endocardial linings. The valve cusps were thin and pliable. The valve orifices were not remarkable. The coronary arteries presented a few fatty intimal plaques, but they were freely patent and their walls thin. The myocardium was pink and firm.

The cranial cavity, the brain, the thyroid gland, the pleural cavities, the lungs, the peritoneal cavity, the gastrointestinal tract, the liver, the spleen, the pancreas, the adrenal glands and the kidneys were not remarkable. There was slight dilatation of the left ureter and renal pelvis, but the ureter opened freely into the bladder. No source of obstruction could be found. The right renal pelvis and ureter were not dilated. The bladder was not remarkable, and the prostate was not enlarged.

Histologic Examination.—A transverse strip of the entire aneurysmal wall was taken from its widest diameter, cut in serial blocks, embedded in paraffin and sectioned. A longitudinal strip extending from the aortic valve cusps to the uninvolved portion of the aorta was treated similarly. Sections from each block were stained with hematoxylin-eosin, by Verhoeff's method for elastic tissue and by a modification of Masson's trichrome stain. Sections taken from the great vessels of the neck, at their roots, and from the thoracic aorta were similarly

stained. Sections from the brain, the heart, the lungs, the liver, the spleen, the pancreas and the kidneys were stained with hematoxylin-eosin.

Striking medial degenerative changes were apparent in all sections of the wall of the aneurysm. The mildest change consisted in an accumulation of homogeneous anuclear material separating the medial fibers and staining pale blue with hematoxylin-eosin. In many situations, this was abundant, covering wide irregular zones, with replacement of medial elements. In such zones, degenerating nuclei of medial muscle fibers were seen, and pinkish sarcoplasmic remnants blended into the accumulated bluish material. In some areas this material formed irregular elongated intercommunicating lakes, sharply demarcated from the surrounding medial tissues. Where the bluish material had escaped, presumably during fixation and dehydration, irregular clefts and cracks remained in the aortic wall.

In the noncystic areas the aneurysmal wall showed varying degrees of disorganization and fibrosis. Varying quantities of medial musculature were recognizable, but in the least involved areas the muscle fibers blended into zones of pinkish hyaline fibrosis. Elsewhere no muscle fibers remained, the whole media being converted into a dense mass of fibrous tissue. The elastic tissue elements were extremely degenerated. Changes in these fibers ranged from a simple partial loss of tinctorial properties, combined with swelling and blurring of outline, to fraying and fragmentation. They had completely vanished, save for a few coiled remnants, in many situations. Scattered through the media were a few groups of mononuclear cells resembling lymphocytes.

The intima showed mild hyaline anuclear thickening for the most part and was indistinctly demarcated from the media. In the oval area noted on gross examination there were atheromatous deposits, characterized by collections of lipid-filled macrophages and cholesterol clefts. Plaques of calcium were present in the deeper portions of the thickened intima in this area. The adventitia was everywhere markedly thickened by dense fibrosis. The fibrous tissue merged into the fibrotic media and also spread outward for some distance. A few adventitial arterioles were surrounded by small collections of lymphocytes. These changes were confined to the wall of the aneurysm. Sections from the great vessels of the neck and from the thoracic aorta showed no lesions. Sections from the other organs revealed nothing of note.

<div style="text-align:center">COMMENT</div>

It is logical to assume that the severe cystic medial degenerative changes described were the cause of weakening of the aortic wall and resultant aneurysmal dilatation. Syphilitic disease of the aorta can be excluded as an etiologic factor on the basis of the negative Kahn test of the blood, the absence of a history of syphilitic infection, the age of the patient and the gross and the microscopic appearance of the aortic lesion. Similarly, arteriosclerosis appears to have had no part in the causation of this aneurysm, since in only one circumscribed area was there evidence of the fatty intimal deposits and calcification seen in aortic arteriosclerosis. Furthermore, arteriosclerosis of a sufficient degree to allow such marked aneurysmal dilatation must be extremely rare, if not unknown, in a subject of this age. Although cystic medionecrosis of the aorta usually occurs in old age and in association with

dissecting aneurysm, Rottino [2] has collected 5 cases from the literature in which the patients were between 21 and 29 years, and has reported 1 case in which, in a woman of 70, the aorta dilated but did not rupture.

The gross and microscopic postmortem findings gave no clue to the cause of the cystic aortic medionecrosis. The earliest lesions showed a homogeneous bluish material accumulating between medial fibers, without evidence of inflammation. This would seem to indicate an essentially degenerative condition. The fact that small numbers of lymphocytes were present in the adventitia can scarcely be adduced as evidence of a primarily inflammatory process. Review of the clinical history reveals no factor of obvious clinical significance. It is true that, although the patient could not recollect it, he may have suffered from one of the acute infectious diseases of childhood. Shennan,[3] among others, has stressed the importance of such diseases in the production of medial "faults" of the aorta. It cannot be denied, therefore, that the lesions in this case may have been related to an acute infectious disease, but no positive evidence in support of this is forthcoming. That trauma had some etiologic significance is likewise not susceptible of proof and seems unlikely. The genesis of the aortic lesions, therefore, in this case as in others, remains unknown.

SUMMARY

A case is reported of severe cystic medionecrosis of the aorta of a young man which produced a large fusiform aneurysm of the aortic arch, with death resulting eventually from a massive intrapericardial hemorrhage that originated from a small perforation in the wall of the aneurysm.

2. Rottino, A.: Arch. Path. **27**:320, 1939; **28**:1, 1939.

3. Shennan, T.: Medical Research Council, Special Report Series, no. 193, London, His Majesty's Stationery Office, 1934.

GUMMA OF THE CORONARY ARTERY, MYOCARDIAL INFARCTION AND GUMMA OF THE HEART

SIDNEY D. KOBERNICK, M.D.

MONTREAL, CANADA

STENOSIS of the ostiums of the coronary arteries is a common result of syphilitic aortitis, but syphilis of the coronary arteries beyond the ostiums is not often observed, while gumma of the coronary artery is still more rarely encountered. Infarction of the myocardium as a result of any of these lesions is exceptional. It follows, then, that a gumma of the coronary artery resulting in myocardial infarction is rare indeed. Such a case has recently come under observation and is now reported.

REPORT OF A CASE

A 44 year old white man presented himself at the medical outpatient clinic of the Royal Victoria Hospital on March 20, 1944 with complaints of ten to fourteen days' duration. They consisted of weakness and dizziness on standing, blurring of vision, buzzing of the ears, cold sweats and dyspnea at rest. On the day of his first examination he had had a severe attack of epigastric pain and numbness of the right leg.

He was seen by Dr. S. Eidlow, who gave me permission to use the clinical records. Dr. Eidlow found the pupils irregular and sluggish, the heart enlarged to the left, with an aortic diastolic murmur heard in the fourth left interspace, and the blood pressure 110 systolic and 90 diastolic. The urine contained albumin, and there was electrocardiographic evidence of a posterior myocardial infarct. He made the diagnosis of syphilitic aortitis with aortic insufficiency and acute myocardial infarction. The Wassermann reaction, reported on March 31, was 4 plus. Immediate hospitalization was recommended.

The patient was admitted to the Royal Victoria Hospital on April 1, where it was elicited that he had had an attack of blurred vision and palpitation of the heart two years before and that he had had malaria in his youth and gonorrhea two years prior to admission, but he denied that there had been any primary syphilitic infection.

The physical examination revealed a well developed man in obvious distress. He was markedly dyspneic and complained of pain in the left part of the epigastrium and the left upper quadrant of the abdomen. His temperature was 97.6 F., pulse rate 90 per minute and respiratory rate 30 per minute; his blood pressure was 110 systolic and 70 diastolic. The heart was enlarged 2 fingerbreadths beyond the left nipple line. The cardiac sounds were distant. A presystolic murmur was heard at the apex, together with a blowing systolic murmur transmitted to the axilla. The aortic second sound was obscured. Gallop rhythm was thought

to be present. The liver was palpable and tender. Tenderness was noted in the left upper quadrant of the abdomen.

The laboratory findings were as follows: The urine had a specific gravity of 1.023 and showed albumin (4 plus), occasional hyaline casts and 2 to 3 pus cells per high power field. The blood showed 9,200 white blood cells per cubic millimeter; the hemoglobin was 76 per cent; the erythrocyte sedimentation rate was 39 mm. in the first hour.

He was placed at complete rest in bed and given sedatives. He became restless and, following a severe attack of pain and dyspnea, died the day after admission.

Postmortem Examination.—This was confined to the abdomen and the thorax and was performed by Dr. E. G. Hinds fifty-six hours after the patient's death. Only the findings directly referable to the cardiovascular system need be described in detail.

The pericardial cavity contained 500 cc. of clear, greenish brown fluid. The pericardium was smooth and glistening throughout. The pulmonary aorta, opened in situ, revealed no embolism.

The heart weighed 645 Gm. All the chambers were somewhat dilated. The myocardium was markedly hypertrophied, the right ventricular wall measuring 4 mm. in thickness and the left 23 mm. On the posterior aspect of the left ventricle, toward the base, was an area measuring 4 by 5 cm. which was mottled brownish yellow as seen through the epicardium. The corresponding endocardial surface had the same appearance. The posterior aspect of the septum near the base was also involved. A thick, firm mass could be seen extending from the region of the orifice of the right coronary artery into the right auricle. The mass was whitish, opaque and nodular, bulging into the auricle above the tricuspid valve. The whole lesion measured about 2 cm. in diameter and consisted of three or four contiguous nodular masses, each about 7 to 8 mm. in diameter. The endocardium was intact over these masses. In the dorsal wall of the pulmonary conus, another mass was seen, similar in size, shape and consistency to the one just described. Between the two lesions a degree of continuity could be demonstrated by palpation. The columnae carneae and the papillary muscles of the left ventricle were markedly hypertrophied. The tricuspid, pulmonic and mitral valves were not abnormal in any way. The aortic valve ring was dilated, measuring 10.5 cm. in circumference. The cusps showed moderate thickening at the line of closure and separation of the cusps for a distance of 2 or 3 mm. at the commissures.

The orifice of the right coronary artery was markedly stenosed to pinpoint diameter. Immediately distal to the orifice, for about 2 cm., the wall of the artery was eccentrically thickened by whitish gray tissue occupying the media and the adventitia. The lumen was small and filled with reddish brown thrombus material. Distal to this, again, the lumen was patent, the intima being involved by arteriosclerosis to a minimal degree. The right coronary artery extended farther over the base of the left ventricle than usual. The ostium of the left coronary artery was moderately stenosed, and no gross abnormality except for a minimal amount of arteriosclerosis was detected in the main trunk or any of its branches.

The aorta showed loss of elasticity throughout, especially in the arch and the ascending and descending thoracic parts. The intima was wrinkled, and between the wrinkles the aortic wall was thickened by grayish blue plaques. The ascending portion and arch presented a fusiform dilatation, 6 cm. in diameter. Two centimeters beyond the orifice of the left subclavian artery, on the anterolateral aspect

of the main fusiform aneurysm, there was a smaller, saccular aneurysm filled with thrombus material. The space between the two aneurysms was occupied by a white, firm, fibrotic, homogeneous mass. This mass projected into the pulmonary artery in such a manner as to cause a bulge in the endothelial surface. It was 1.5 cm. in diameter and nodular and ovoid. The left common carotid artery was narrowed for a short distance from its origin in the dilated aortic arch. The other two main branches of the arch of the aorta appeared normal. The medium-sized arteries were not otherwise unusual.

Microscopic Examination.—The right coronary artery, near its origin, presented marked thickening of the adventitia by a dense layer of collagenous tissue containing many capillaries surrounded by lymphocytes and plasma cells. Just
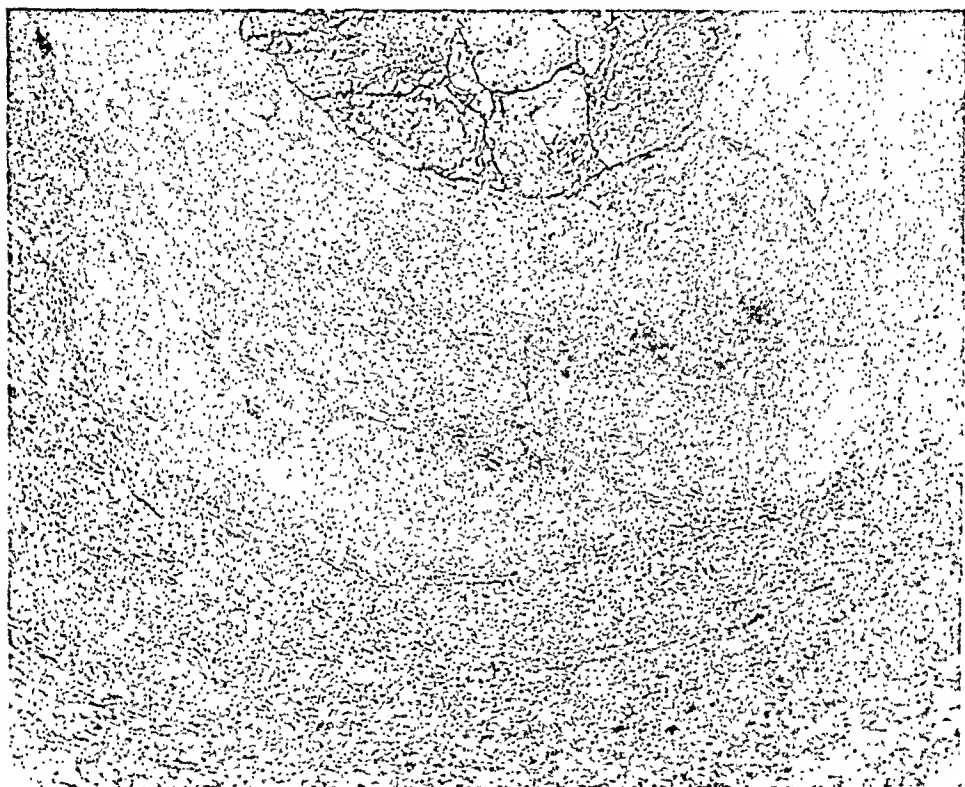
Photomicrograph of a section of the right coronary artery showing an occlusive thrombus, gummatous necrosis of the wall, an adventitial reaction and fibrosis. Hematoxylin and eosin; × 34.

inside this was a zone of loose vascular connective tissue diffusely infiltrated by lymphocytes and plasma cells, which surrounded the media. The media was necrotic throughout its whole thickness, and the necrosis extended into the inner layers of the intima. The necrosis was of the coagulative type, ghost structure of the media being present in some places. The intima was greatly thickened by rather dense hyaline connective tissue, which reduced the lumen to approximately half its normal diameter. The lumen was occluded by a thrombus (figure). Sections just distal to this one, in the artery, showed marked fibrosis of the adventitia, which was very dense in most places. Many arterioles of the adventitia showed pronounced endarteritis obliterans. There was considerable lymphocyte and plasma

cell infiltration throughout the adventitia. The elastic tissue of the media was considerably distorted in some areas by ingrowth of vascular granulation tissue, in which there was moderate lymphocytic infiltration.

Six sections were taken from various parts of the distal portions of the left and right coronary arteries, and a few of these sections showed moderate intimal arteriosclerosis, more marked in the larger than in the smaller distal portions. The adventitia showed no excess fibrosis or infiltration. The media was, for the most part, uniform in thickness except in the more markedly arteriosclerotic areas, where it was somewhat atrophic.

Sections of the myocardium showed in the area of infarction large areas of necrotic muscle fibers in which there was hemorrhage and some infiltrating white blood cells, which were also degenerating. Into this was growing vascular granulation tissue, and in some areas small numbers of necrotic muscle fibers had been replaced. However, organization was in an early stage.

Microscopic examination of other areas of the heart showed only hypertrophy of muscle fibers with minimal interstitial fibrosis. The lesion between the pulmonary artery and the aorta had the histologic appearance of a gumma. The lesion in the right ventricle was also a typical gumma. Sections of the aorta showed extensive syphilitic involvement with endarteritis obliterans of the vasa vasorum, perivascular cuffing by lymphocytes and plasma cells, medial necrosis and fibrosis and hyaline intimal thickening.

COMMENT

This, then, is a case of posterior myocardial infarction following thrombotic occlusion of the right coronary artery. The sequence of events indicated by the findings was syphilitic arteritis beyond and separate from the process in the aorta, followed by gummatous necrosis of the arterial wall, which precipitated thrombosis and which was followed, in turn, by infarction of the myocardium in the area supplied by the artery. Typical gummas were found in the myocardium, projecting into the right auricle and the pulmonary conus. The pathologic observation of recent thrombosis and infarction of only a few days' duration was consistent with the clinical picture.

Stenosis and occlusion of the ostiums of the coronary arteries have been frequently reported, with an incidence varying from 12 to 8 per cent of cases of syphilitic aortitis.[1] Syphilis involving the coronary arteries beyond the ostiums, though much less frequent, has been described by Moritz[2] and Maher[3] and has been estimated by Cormia[1f] to occur in 25 per cent of cases of aortitis in which the ostiums were involved. When it is present, it is said to be in the first 10 or 12 mm.,[2] though it is also reported to occur in the more peripheral portions.[4] Thrombosis

1 (a) Bruenn, H. G.: Am. Heart J. **9**:421, 1934. (b) Burch, G. E., and Winsor, T.: ibid. **24**:740, 1942. (c) Carr, J. G.: ibid. **6**:30, 1930. (d) Clawson, B. J., and Bell, E. T.: Arch. Path. **4**:922, 1927. (e) Clawson, B. J.: Urol. & Cutan. Rev. **45**:219, 1942. (f) Cormia, F. E.: Canad. M. A. J. **33**:613, 1935. (g) Martland, H. S.: Am Heart J. **6**:1, 1930. (h) Saphir, O., and Scott, R. W.: Am. J. Path. **3**:527, 1921; (i) Am. Heart J. **6**:56, 1930. (j) Saphir, O.: Arch. Path. **13**:266 and 435, 1932. (k) Warthin, A. S.: Am. Heart J. **6**:163, 1930,

2. Moritz, A. R.: Arch. Path. **11**:44, 1931.

3. Maher, C. C.: Am. Heart J. **6**:37, 1930.

4. Warthin, A. S.: J. A. M. A. **84**:1597, 1925. Maher.[3]

associated with syphilitic involvement of the coronary arteries has not been described. Nor is there any case recorded in the literature in which a gumma of a coronary artery precipitated thrombosis and infarction as in the case reported here. The only gumma of a coronary artery mentioned in the literature [1k] was not followed by thrombosis or infarction. However, cases of myocardial infarction due to syphilis affecting the coronary arteries in other ways have been reported, such as those due to syphilitic occlusion of the coronary ostiums without thrombosis described by Corrigan,[5] Cowan and Rennie,[6] Burch and Winsor,[1b] Bruenn[1a] and Saphir[1j]—10 cases in all. Chipps[7] reported a case in which the left coronary artery was compressed by a syphilitic aneurysm of the sinus of valsalva, and Snyder and Hunter[8] reported a similar aneurysm which caused thrombosis and infarction. There are also cases of *infarction on the basis of arteriosclerosis associated with syphilis.*[9]

The infrequency of myocardial infarction due to syphilitic involvement of the coronary arteries is generally attributed to the slowness of the process, which allows collateral circulation to develop to a sufficient degree to prevent acute ischemia of the myocardium. That this can occur to an extreme degree is evident from Wearn's[10] 2 cases, in which there was complete occlusion of both coronary ostiums without myocardial infarction having occurred. In those cases the circulation of the heart must have been maintained entirely by means of extracardiac anastomoses.[11]

Gumma of the myocardium is also a rare lesion. Sohval[12] found 97 cases of gumma of the myocardium recorded in the literature up to 1935. In these cases every part of the heart was the site of a gumma, but in none did a gumma involve a coronary artery. Eleven cases of gumma of the heart have been reported in the literature since 1935.[13]

SUMMARY

An unusual case of gumma of the heart and gumma of the right coronary artery resulting in thrombosis of the arterial lumen and infarction of the myocardium is reported.

No similar case has been previously described in the literature. Gumma of the heart is itself rare, only 108 cases being reported in the literature. In only 1 instance has gumma of the coronary artery been described, but no mention was made of thrombosis or infarction having occurred.

5. Corrigan, M. C.: Urol. & Cutan. Rev. **45**:229, 1941.

6. Cowan, J., and Rennie, J. K.: Brit. M. J. **2**:184, 1921.

7. Chipps, H. D.: Arch. Path. **31**:627, 1941.

8. Snyder, G. A. C., and Hunter, W. C.: Am. J. Path. **10**:757, 1934.

9. Corrigan.[5] Warthin.[1k]

10. Wearn, J. T.: J. Exper. Med. **47**:293, 1928.

11. Hudson, C. L.; Moritz, A. R., and Wearn, J. T.: J. Exper. Med. **56**:919, 1932.

12. Sohval, A. R.: Arch. Path. **20**:429, 1935.

13. von Haam, E., and Ogden, M. A.: Arch. Path. **26**:525, 1938. Spain, D. M., and Johannsen, M. W.: Am. Heart J. **24**:689, 1942. Pratt-Thomas, H. R.: ibid. **36**:80, 1943. Clawson and Bell.[1d]

General Reviews

MECHANISMS OF ABNORMAL DEVELOPMENT

II. Embryonic Development of Malformations

PETER GRUENWALD, M.D.

NEW YORK

MOST treatises on abnormal development classify their subject either according to morphologic aspects or according to hypothetic modes of origin deduced from the final structure as, for example, inhibition or excess of development and fusion or fission of primordia. Cyclopia, for instance, would be found in the category of malformations by fusion of normally separate bilateral primordia. The brief discussion of cyclopia given in the introduction to the first part of this review shows that this or any other classification of it under the system just mentioned must be incorrect and misleading. Many other malformations develop in a manner which cannot possibly be detected by the study of the final condition alone as, for instance, the defects of the extremities in a hereditary syndrome of malformations in mice ("myelencephalic blebs," see page 556). It thus appears that a system based on hypothetic mechanisms of development of malformations is of no value either for classification. or as a guide for future investigations. Its inadequacy has been pointed out by Weiss.[3] The causes of malformations, on the other hand, do not constitute a suitable basis for classification for two reasons. One is that in cases of malformation not produced in the laboratory the cause is often unknown, and the other reason is that many different causes may produce similar effects, or the same cause different effects under but slightly differing conditions. This leaves, in the present state of knowledge, only a classification from the standpoint of morphology, and that will not be discussed here as this presentation is not concerned with purely morphologic aspects of the subject.

In the following pages some of the better studied mechanisms will. be described by which a malformation develops into its final form after an initial lesion has been produced by a known or an unknown cause.

Grüneberg [11] classifies the mechanisms by which mutations manifest themselves as structural abnormalities, as follows:

(*a*) A normal developmental process is suppressed or remains incomplete.

(*b*) A normal developmental process is exaggerated.

(*c*) A normal developmental process is deflected in the wrong direction.

(*d*) The changes are regressive (degenerations).

Grüneberg admits that there are no clearcut borderlines between these mechanisms and that some abnormalities could conceivably be classified under different headings. He also suggests that it may be necessary to reclassify a malformation if new information is obtained regarding its development. Many of the nonhereditary malformations can also be classified in this manner.

A more detailed classification, which also includes mechanisms occurring in malformations due to extrinsic factors, will now be given:

(*a*) Inhibition or excess of a developmental process.

(*b*) Abnormal developmental pattern (absence, excessive number, division, fusion or abnormal location of primordia).

(*c*) Qualitatively abnormal development.

(*d*) Degeneration of previously normal-appearing parts.

(*e*) Interference by nonspecific reaction to injury.

(*f*) Elimination of parts.

The basic abnormal process is usually limited to a short period of development, leaving a part of the body in an abnormal condition. The affected part will thereafter develop by normal mechanisms but will show the effect of the interference because these normal mechanisms occur in an abnormal substrate. In some cases the abnormality may be more or less eliminated by regenerative processes.

Syndromes of malformations occur when the primary abnormality affects more than one part, or when the primarily affected part influences other parts so that they, too, become abnormal. The correlations which link the various manifestations of teratologic syndromes may be of the following kinds:

(*a*) Genetic mechanisms.

(*b*) Equal susceptibility of parts.

(*c*) Developmental patterns governing several parts.

(*d*) Functional dependence.

(*e*) Mechanical correlations.

These mechanisms are by no means mutually exclusive. In fact, few instances will be found in which only one of them is in effect. They

can therefore not be used for a classification of fully developed syndromes but are used rather as guides for the study of the development of the syndromes. It must be remembered that some of these mechanisms normally safeguard the development of proper morphologic relations of parts, but they may greatly increase the extent of a malformation if they are shifted to wrong tracks, as it were, by an abnormality somewhere along the line of developmental steps. These chains of events explain the occurrence of syndromes of malformations and the often puzzling repetition of complex developmental aberrations in many persons. The following examples of mechanisms of developmental correlation are given to illustrate the aforementioned types.

Genetic Correlations.—When several malformations which are independent of·each other in their development are caused by one gene they may be said to be genetically correlated. Grüneberg [232] holds that this so-called pleiotropic gene effect does not exist and·that an adequate understanding of the developmental mechanism would in all instances reveal a single primary developmental disturbance responsible for all manifestations. It is true that in several instances complex hereditary syndromes of malformations have been traced back to a single initial disturbance. Gluecksohn-Schoenheimer [233] has recently criticized Grüneberg's view and holds that final judgment should be reserved until more is known about gene action. On purely speculative grounds one may say that since it is assumed that each gene is uniform, its primary action is likely to be uniform as well. Whether or not one accepts the concept of pleiotropic gene action on development depends on the arbitrary decision whether or not the primary action is considered a developmental process. Examples of apparently pleiotropic gene effect are instances of hereditary intersexuality in various mammals associated with a peculiar color of coat [59a] or with skeletal malformations.[234]

Another possibility of genetic correlation is linkage of genes which are located at nearby points in the same chromosome so that it is improbable that they will be separated during the maturation of germ cells. The results of numerous linkage tests in the mouse have been reviewed by Snell [1p] and Grüneberg,[1r] and recently in the form of a chromosome map.[235] If a gene is located in the sex chromosome, its transmission is modified by the peculiarities of that chromosome: sex-linked inheritance. In the present discussion of the embryogenesis of malformations, genetic correlations will not be considered, since their action is not on

232. (*a*) Grüneberg, H.: J. Genetics **45**:1, 1943; (*b*) footnote 1r.

233. Gluecksohn-Schoenheimer, S.: Genetics **30**:29, 1945.

234. Surrarrer, T. C.: J. Hered. **34**:175, 1943.

235. Staff of the Roscoe B. Jackson Memorial Laboratory: J. Hered. **36**:271, 1945.

a developmental basis, but precedes development. Only their results will occasionally be referred to.

Equal Susceptibility of Parts.—It must be recognized that equal susceptibility of primordia to the action of teratogenic factors of any kind is an important mechanism by which seemingly unrelated developmental processes may be affected by one agent. It was reported in part I that many workers in the field of teratology consider the majority of teratogenic stimuli as nonspecific retardations of metabolism and development, which have a seemingly specific effect on certain parts only because these parts are highly susceptible at the time of their action. This susceptibility is thought to be greatest while a given part is growing or differentiating rapidly, and if this is true, it is obvious that several parts may be in a highly susceptible phase at the same time. This has been emphasized in the explanation of multiple malformations in various parts of the body caused by the Px mutation in guinea pigs.[236]

Developmental Patterns Governing Several Parts.—Correlations determined by the developmental pattern exist when an abnormal pattern of a larger portion of the body determines the development of malformations in several parts, much in the same manner in which the normal pattern would determine normal development of the same area. In cyclopia, for instance, a change in the developmental pattern of the neural plate is responsible for malformations of brain and eyes.

Correlation by Independent Development.—This occurs in abnormal development just as in normal ontogenesis. If one part depends on another for its normal development, it will be abnormal if that other part is absent or abnormal. Thus absence of a wolffian duct in the embryo will result not only in absence of the ductus deferens, which arises from it, but in absence of the epididymis and the kidney because these organs depend in their development on stimulation by the wolffian duct and its branch, the ureteric bud, respectively.

Functional Dependence.—Correlation by functional dependence may occur in various forms. Defects of the respiratory organs of the embryo may produce malformations of various organs by anoxia before, or without, killing the embryo. This occurs in the homozygous Creeper chick embryo when the circulation of its yolk sac fails (see page 554). Abnormal function of endocrine organs may have profound effects on development as, for instance, excessive hormone production by adenomas of the adrenal cortex, which promotes sex reversal in the embryo as well as after birth. Correlations between the nervous system and the organs supplied by it are conspicuous in postnatal life and not entirely absent in the embryo.

---

236. Scott, J. P.: (*a*) J. Exper. Zool. **77**:123, 1937; (*b*) J. Morphol. **62**:299, 1938.

Mechanical Correlations.—If an abnormal condition of one part makes it mechanically impossible for another part to pursue its normal course of development, even though its primordium is present and normal, there is observed a mechanical correlation affecting development. The just mentioned absence of a wolffian duct, for instance, has 'a mechanical effect in addition to the one already noted. The duct serves normally as a mechanical guide of the müllerian duct, which grows caudad within the wolffian duct's basal membrane. In the absence of this guide the müllerian duct fails to grow, and as a result the tube and one horn of the uterus are absent if the individual is of female sex. Another example is the absence of a choroid fissure in maldeveloped eyes, as it occurs in certain cases of microphthalmia or cyclopia (see page 518). It deprives the optic nerve fibers of their passageway to the brain, and results in absence of the optic nerve even though the optic stalk and the nerve fibers are all originally present.

The foregoing may not be a complete listing of all the methods by which abnormal traits, once appearing at one point, may express themselves throughout the organism, but it comprises the essential mechanisms found in the malformations to be described. It is obvious that these correlations may appear in various combinations, as may be gathered from the fact that several of the few syndromes of malformations given as examples appear under more than one heading.

Malformations appear in nature and in the laboratory with varying degrees of regularity, and this has been used to classify them as typical or atypical.[237] Malformations of the former type are found repeatedly either as identical forms or as various degrees of the same type of aberration. They appear to follow a plan almost or wholly as definite as that of normal development. Atypical malformations, on the other hand, are irregular and unpredictable. The typical malformations are supposedly due to genetic causes, the atypical ones to environmental influences which may attack the embryo at any time and place. The latter statement is not correct, as is illustrated by the regular production of malformations by influencing the embryo itself (see the first part of this review) and particularly by the existence of phenocopies in which a genetically caused malformation is copied by interference with ontogenesis alone. However, it is obvious that some of the aforementioned types of correlation of abnormal developments will tend to yield typical, and others atypical, defects. Genetic correlations of abnormal traits are as regular as those governing normal development, and they yield consistent results unless other, modifying genes or extrinsic agents interfere. Syndromes caused by abnormal developmental patterns or dependent

---

237. von Szily.[1c] Politzer, G., and Sternberg, H.: Frankfurt. Ztschr. f. Path. **37**:174, 1929.

development will be among the most regular ones. On the other hand, the most irregular and atypical abnormalities may be found among simple malformations produced by extrinsic agents.

Another factor which may influence the final appearance of a structural abnormality is restitution of defective parts. In embryonic malformations the normal mechanisms of regulation of development will often provide some restitution of a defective pattern before it is structurally differentiated, and this will then not be apparent as regeneration. There are extensive studies of regeneration of extremities in larvae of amphibia [238]; their results have contributed materially to the understanding of malformations of these parts. An interesting example of the complicated mechanisms of regeneration is that of replacement of an extirpated lens in amphibia, the so-called wolffian lens regeneration. The new lens is formed not from the superficial ectoderm (cornea) as was the normal lens but from the upper portion of the iris, which is normally not concerned with lens formation.[239] In malformations of the eyes, abortive or even well organized lenses may develop in the same manner.[18] Schotté [240] has presented a concise review of the general problems involved in regeneration.

In the following pages some of those malformations on the embryonic development of which information is available will be discussed. Then the widespread manifestations of two extensively studied hereditary syndromes of malformations (Creeper, myelencephalic blebs) will be summarized, as well as the effects of heterospecific pregnancy.

TWINS AND DOUBLE MONSTERS

Of the two principal types of twins and multiple births, the dizygotic or fraternal twins are of little interest in the present discussion since they are not essentially different from brothers and sisters born at different times except that they may, on rare occasions, interfere with each other's development. This occurs particularly if close relations develop between the chorions of the siblings, leading to fusion of the membranes and often to anastomoses of their circulatory systems. This fusion is known to occur in man [241] as well as in other mammals.[242] In certain species of mammals, anastomoses between the circulations of heterosexual dizygotic twins result in abnormal sexual development of the female, apparently caused by some substance transmitted from the male partner. In other species, including man, no such influence is ever exerted in the presence of vascular anastomoses (see part I).

238. Mangold, O.: Ergebn. d. Biol. 5:290, 1929. Weiss.[3] Needham.[4]
239. Mangold.[1f] Weiss.[3]
240. Schotté, O. E.: Growth (supp.) 3:59, 1939.
241. Arey, L. B.: Anat. Rec. 23:253, 1922.
242. Witschi.[160] Wislocki, G. B.: Am. J. Anat. 64:445, 1939.

Monozygotic or identical twins, arising from a single, normally fertilized egg cell, are of great interest from the point of view of teratology. While each partner of the pair may be perfectly normal, monozygotic twinning in man is too rare to be considered as a normal occurrence. In certain animals, on the other hand, it occurs invariably and must be regarded as normal. The close relationship of twins to double monsters will be discussed subsequently. In the laboratory, twins and double monsters have been produced by a variety of methods. A thoroughly investigated procedure is that of constricting amphibian eggs to a varying extent, which results in separate twins or monsters with varying degrees of duplication depending on the intensity of constriction.[2] Many other methods have yielded twins and double monsters by mechanisms which are not so well understood, such as hybridization, delayed fertilization,[36] irradiation with roentgen [16a] and ultraviolet rays,[85] splitting,[243] centrifuging [72] or chemicals.[244] A hereditary influence in human twinning is assumed by numerous authors [245] and denied by others.[246] An example of definite hereditary twinning is the development of multiple embryos in germs homozygous for the lethal gene "kinky" which in heterozygous condition produces abnormalities of the vertebrae, the tail and the labyrinth. These germs die early.[247] Danforth [248] described hereditary duplication of the caudal part of the body in mice.

There has been much speculation concerning the cause of polyembryony (monozygotic multiple birth) as it occurs regularly in the armadillo, partly with a view to explaining human twinning. Newman [249] and Stockard [1n] hold that a temporary slowing of development, which is known to occur in these species, is responsible for a dispersion of the organization center into several independent centers; these authors also suggest that cooling of birds' eggs before gastrulation (which normally occurs before laying) is responsible for twinning. Sturkie [250] found that if hens are chilled about the time of ovulation and cleavage, the incidence of double embryos rises to 8.2 per cent. Newman set up a hypothesis for polyembryony of the armadillo, which includes the following steps:

243. Morita, S.: Anat. Anz. **82**:81, 1936; **84**:81, 1937. Twiesselmann, F.: Arch. de biol., Paris **49**:285, 1938.

244. Werber.[99] Morita.[243]

245. (*a*) Danforth, C. H.: J. Hered. **7**:195, 1916. (*b*) Davenport, C. B.: Proc. Soc. Exper. Biol. & Med. **17**:75, 1920. (*c*) Wehefritz, E.: Ztschr. f. menschl. Vererb- u. Konstitutionslehre **11**:554, 1925. (*d*) Hamlett, G. W. D.: Anat. Rec. (supp. 2) **73**:26, 1939.

246. Greulich, W. W.: Am. J. Phys. Anthropol. **19**:391, 1934.

247. Gluecksohn-Schoenheimer, S.: Anat. Rec. **94**:462, 1946.

248. Danforth, C. H.: Am. J. Anat. **45**:275, 1930.

249. Newman, H. H.: The Physiology of Twinning, Chicago, University of Chicago Press, 1923.

250. Sturkie, P. D.: J. Exper. Zool. **101**:51, 1946.

slow formation of the corpus luteum; no response of the uterine mucosa to the early embryo; late placentation; cessation of development for three weeks; deaxiation of the embryo; isolation of four growing regions. Hamlett [251] opposes this view and assumes that only genetic control, and not differences in temperature or in metabolic rate, can account for a multiplicity of organization centers that occurs as regularly as that seen in the armadillo. In support of this he [252] quotes the familial occurrence of twinning in man and the fact that many mammals regularly show a standstill of early embryonic development without twins ever developing. These arguments do not completely disprove the first mentioned hypothesis, as hereditary factors in man may be thought to produce twinning by reducing the metabolic rate at a given point, and the slowing of development in species without twinning may not occur just at the time when the organization center is established (of which time investigators have no knowledge). It is known that minute differences in the timing of teratogenic action may completely change the result (see part I). The assumption of cooling as the cause of twinning in birds is opposed by Riddle [253] on experimental grounds. Needham [4] tends to agree with Hamlett's view. Arey [254] finds an increased incidence of monozygotic human twins in ectopic pregnancies and is therefore inclined to assume the existence of extrinsic factors. Here, as in many other teratologic arguments, it is often forgotten that many different agents, hereditary and environmental, may produce the same result and that the occurrence of one agent in some cases does not disprove the presence of others in other cases.

The mutual relations of monozygotic twins and their membranes depend on the stage at which twinning occurs. According to Greulich,[216] all three theoretically possible forms may occur: a fertilized egg cell may divide and the daughter cells separate themselves from one another and develop into twins with completely separated membranes and placentas. If twinning manifests itself later in development, a single blastocyst may develop but may contain two embryoblasts resulting in a common chorion and separate amnions. Finally, from a single embryoblast in a single blastocyst two embryos may develop in one embryonic disk, resulting in twins with a common amnion and chorion, as well as a common yolk sac.[254] If all these possibilities should concur, monozygotic twins could have all possible relations of their membranes, including separate chorions, which are often assumed to be proof of dizygotic origin. This, together with the fact that the chorions of dizy-

251. Hamlett, G. W. D.: Quart. Rev. Biol. 8:348, 1933.
252. Hamlett, G. W. D.: Quart. Rev. Biol. 10:432, 1935.
253. Riddle, O.: Am. J. Anat. 32:199, 1923.
254. Arey, L. B.: Anat. Rec. 23:245, 1922.

gotic twins may fuse secondarily into one, shows that the membranes cannot be relied on in the determination of the type of twinning in a given case. More and more emphasis is therefore being placed on various hereditary somatic characters in the twins which should be significantly more similar in monozygotic than in dizygotic twins. However, work with these somatic traits revealed intermediate conditions, which led Danforth [245a] to postulate a third type of twinning. In this type only one gamete, which can only be the egg cell, is common to both twins, whereas their paternal component is derived from two different spermatozoons. No definite explanation of the mechanism is given, but the following possibility is suggested on the basis of work on lower animals. Immediately after an egg cell has been penetrated by a spermatozoon it divides in such a manner that only one of the daughter cells copulates with the entire chromatin of the spermatozoon. The other daughter cell is subsequently fertilized by another spermatozoon. This hypothesis has been taken over by Greulich [246] in a somewhat distorted form, according to which each of the daughter cells would finally be triploid, with chromosomes from two spermatozoons. This is an untenable as the old idea that twins derive from an egg cell fertilized by two spermatozoons. Boveri [255] showed long ago that fertilization of an egg cell by two spermatozoons leads not to twins but to early death, owing to unequal distribution of the chromatin of three gametes among the poles of a tetrapolar mitosis during the first cleavage.

The close topographic relations of monozygotic twins may lead to interference with the normal development of one or both partners. If extensive vascular anastomoses exist between the twins, particularly in a common placenta, one twin may become an acardius if its circulation is inferior to that of the other sibling and is finally taken over by the stronger one. The heart of the weaker twin will stop functioning, and the heart of the stronger one will drive the blood through the common placenta and the other twin. The acardius is invariably and severely maldeveloped in the late stage in which it is usually examined; large parts of the body are entirely absent. Two opposing views of the possible development have been advanced,[256] one holding that both twins are normal at first and differ only in vigor, and the other one assuming that the acardius is primarily maldeveloped, and its circulation taken over by the other twin as a consequence of this. Examination of a pertinent case of twins in a very early stage has recently [83] produced a third suggestion, namely, that the future acardius may in itself be normal but have abnormal vascular conections with the other twin and the placenta. In the case in question, as well as in one briefly described

---

255. Boveri, T.: Zellenstudien, Jena, Gustav Fischer, 1907, vol. 6.

256. Schwalbe, E.: Die Morphologie der Missbildungen des Menschen und der Tiere, Jena, Gustav Fischer, 1907, pt. 2.

by Heaney and Bartelmez,[257] one of the twins has no direct vascular conections with the placenta. It is possible that any one of these three mechanisms may occur, depending on the conditions in each case.

Mechanical interference of twins with each other is occasionally observed. The history of the fetus papyraceus is not well understood. In early stages, pressure by a twin may cause invagination of the cranial end of the body into the yolk sac (omphalocephaly); this has been found in several birds.[82] Invagination of the caudal end into the yolk sac (ourentery), probably caused by the other twin, has been seen in a human ovum.[83]

Double monsters are structurally closely related to monozygotic twins. All transitions exist from low degrees of duplicity, which may be almost unnoticeable externally, through the commonly illustrated types of double monsters (conjoined twins) to identical twins which have separated bodies but which have in common one of their most important fetal organs, the placenta. The fact that the last-named twins are really also conjoined has been emphasized by designating them as choriopagi because of their common chorion, in analogy with the term "thoracopagi" for twins fused at their chests. Hamlett[245d] claims that monozygotic twins and double monsters are unrelated, basing his contention on the observation that double monsters do not occur in families with a hereditary tendency toward twining and are not related to inherited tendencies at all. Against this stand not only morphologic considerations but also the findings of Danforth[248] of hereditary posterior duplication in mice. It is doubtful whether statistically significant genetic results can be obtained concerning human double monsters in view of the rarity of the condition and the possibility of inheritance by more than one gene, perhaps in interaction with genetic or environmental modifiers. In Spemann's constriction experiments (see page 501) a variation in the intensity of the same agent produced all transitions from double monsters to twins.

The question has often been raised whether double monsters originate by splitting of one embryonic anlage or by fusion of two.[258] To me this appears immaterial, since it is obvious that at some early stage, probably before the beginning of visible differentiation, a division of the germ into two parts with their own organization centers must have occurred. Whether or not they fuse later on depends largely on topographic relations which, in turn, are partly determined by the time at which the duplicity is determined.

A considerable proportion of double monsters are asymmetric; that is, one of the partners is defective, depends for support on the other and

257. Heaney, N. S., and Bartelmez, G. W.: Anat. Rec. (supp.) 48:47, 1931.
258. Newman, H. H.: J. Hered. 31:371, 1940. Waddington, C. H.: ibid. 32:268, 1941.

is referred to as a parasite. A comparable condition in separate twins is the acardius (see page 503). All grades of intermediate conditions have been described, leading from the relatively well formed parasitic twin to inclusions consisting of irregular mixtures of tissues or organs, namely, teratomas. Various theories of teratomas and their relationship to neoplasms will be briefly discussed later in this review. The occurrence of twins and teratomas in the same families has been observed,[239] and interpreted as indicating a related pathogenesis.

MEDIAN DEFECTS

An indication of the importance of median defects and related problems in the study of teratology was given in the brief review of cyclopia in the introduction to part I of this review. In addition to symmetric median defects of the head, including cyclopia, otocephaly and essentially similar malformations of minor degrees, defects of the caudal portion of the body have been investigated in great detail. Best known among these are sirens with fused legs, but there are many other varieties, mostly of less extent. Some of these are of medical importance, since they are viable or may be so with the help of plastic surgery. ·Finally, there is a small and not so well investigated group of similar defects of the trunk which do not affect either end of the body and will here be referred to as interstitial defects.

The median, symmetric defects of the head are often referred to under the common name of cyclopia (in the wider sense), and this will also be done here unless otherwise indicated. In the strict sense, "cyclopia" means a malformation in which the eyes are fused into one median organ. Several other defects are known to be regularly or frequently associated with this condition, such as a single median nasal cavity in a trunklike nose (proboscis) above the eye, defects of the oral cavity and of the derivatives of the branchial arches, and brain defects. Median defects of greater extent may lead to complete absence of the eyes. There may be ventral approximation or fusion of the ears, otocephaly. On the other hand, less degrees of median defects may leave the ears in their normal locations and the eyes approximated but separate (synophthalmia), or affect only the brain (arhinencephaly).

It is superfluous to describe in detail the development of knowledge of cyclopia as briefly outlined in the introduction, since all work up to 1936 has been reviewed by Adelmann.[260] His view is briefly as follows: In the normal embryo the pattern for the prospective eyes and nearby parts of the brain is determined in the neural plate stage in a labile manner. In the production and maintenance of this pattern the underlying tissue of the roof of the archenteron (the so-called substratum),

259. Edmonds, H. W., and Hawkins, J. W.: Cancer Research 1:896, 1941.
260. Adelmann, H. B.: Quart. Rev. Biol. 11:161 and 284, 1936.

and particularly the prechordal mesoderm, plays a leading role. The regular association of the malformation of the eyes and brain with defects of mesodermal parts in spontaneous and experimental cases indicates that an abnormality of the substratum (which develops into the defects of mesodermal parts) determines an abnormal pattern in the neural plate which in itself may have been normally formed. This was confirmed when cyclopic changes were induced in normal neural plates of amphibian embryos by experimental defects of the substratum alone.[261] It is not believed to be the rule that the primary defect comprises parts of mesoderm and ectoderm alike, although experimental defects of this kind have led to cyclopia.[262] Only minor contributions to the problem of cyclopia have been made since Adelmann's review. Lehmann[263] assumes that among the parts of the substratum the entoderm is more important for the determination of ocular development than the mesoderm. Marburg and Mettler[264] add to older studies of the morphologic aspects of cyclopia a detailed investigation of the nuclei of the cranial nerves. Gruenwald[265] gives an explanation of the frequent absence of the optic nerve in cyclopia, which puzzled previous workers because it could not readily be accounted for by changes in the pattern of the developing eye on the assumption of a primary absence of the optic stalk. In conformity with observations of microphthalmia of chick embryos it is assumed that an optic stalk is present in early stages but degenerates when nerve fibers fail to grow into it because of absence of a choroid fissure. A very recent interpretation of cyclopia[266] discards all previous hypotheses, however well founded, and proposes, on the basis of speculation, a different mechanism. The first aortic arches are assumed to fuse with one another and thus mechanically alter the direction in which the primordia of the eye grow out from the brain, causing them to fuse. Apart from all other difficulties of this explanation, it is obvious that cyclopia cannot develop by fusion of two eyes which are pushed toward each other. It must be determined in the pattern of the formation of eyes previous to the actual outgrowth, and fused aortic arches can have no part in this. It may be of interest to compile the various causes of cyclopia and related defects as observed in the laboratory (see also part I): elimination of tissue by excision or radiation,[8] centrifuging of fertilized eggs,[72] chemical treatment,[267] mutations[11] or hybridization.[43]

261. Adelmann.[9] Mangold.[1f] Sperling, F.: Anat. Rec. **85**:413, 1943.

262. Lewis.[7] Wolff.[8]

263. Lehmann, F. E.: Rev. Suisse de zool. **45**:413, 1938.

264. Marburg. O., and Mettler, F. A.: J. Neuropath. & Exper. Neurol. **2**:54, 1943.

265. Gruenwald, P.: Anat. Rec. **91**:13, 1945.

266. Krafka, J.: Arch. Ophth. **33**:128, 1945.

267. (*a*) Stockard.[6b] (*b*) McClendon.[6d] (*c*) Werber.[100] (*d*) Adelmann, H. B.: J. Exper. Zool. **67**:217, 1934. (*e*) Franke and Tully.[125]

The median symmetric defects of the caudal part of the body have not been investigated as thoroughly as those of the head. This is due largely to the peculiarities of the normal development of that region. There are not, as in the head, several distinct embryonic structures which, in order to develop properly, interact with each other in many ways. There is, on the contrary, a morphologically homogeneous mass of mesenchyme, the trunk-tail node. From this the respective parts of those organ systems arise which develop from distinct germ layers in the head region. This is a formidable obstacle to the experimental investigation of normal and abnormal correlations during early development.

The best known and most conspicuous representatives of caudal defects are sirens which have their legs fused into one median extremity. The skeleton shows parts of both extremities more or less incomplete or fused as, for instance, two femurs, two tibias and one median fibula. The normally lateral (fibular) sides of the extremities are turned medially, owing to changes in the pelvis, and are therefore the first to fuse. The knee points backward. For a review of the morphologic aspects of sirens, see Gruber.[268] Sirenoid malformations, as all caudal median defects have been called, also include minor degrees, such as sacral [269] and lumbosacral [270] defects, anchypodia (approximation and rotation of lower extremities without fusion) and other forms to be mentioned in the following pages. Examination of nonviable early embryos, homozygous for lethal genes, has revealed an instance of median defect severer than siren. In homozygous T mice the entire caudal portion of the body is absent.[271] These embryos die early. The reason why these forms are not usually seen is probably that they lack the allantois and its vessels, which are indispensable for the nutrition and the respiration of the embryo, and therefore die before they can be detected.

Feller and Sternberg [272] first devised a method by which all sirenoid malformations can be uniformly and systematically interpreted and classified. It is basically similar to that of Fischel in that it outlines the malformations in hypothetic early stages as wedge-shaped defects varying in length, width and position. This concept has been widely accepted, and Feller and Sternberg [273] extended it to certain defects of pelvic organs

268. Gruber, G. B., in Schwalbe,[256] 1937, pt. 3, p. 557.

269. Berman, W.: Am. J. Obst. & Gynec. **50**:447, 1945.

270. Zeligs, I. M.: Arch. Surg. **41**:1220, 1940. Sinclair, J. G.; Duren, N., and Rude, J. C.: ibid. **43**:473, 1941.

271. Chesley, P.: J. Exper. Zool. **70**:429, 1935. Gluecksohn-Schoenheimer, S.: Proc. Nat. Acad. Sc. **30**:134, 1944.

272. Feller, A., and Sternberg, H.: Virchows Arch. f. path. Anat. **280**:649, 1931.

273. Feller, A., and Sternberg, H.: Ztschr. f. Anat. u. Entwcklngsgesch. **108**:283, 1938.

without abnormalities of the extremities, such as some forms of atresia ani and absence of the external genitalia. Klaften and Politzer [274] applied similar considerations to certain malformations with a persistent cloaca (a common terminal portion of the digestive and urogenital tracts). It is understood in all these considerations that, as in the corresponding concept of cyclopia, the defect is never present as a gap resulting from elimination of tissue but rather is present as a change which causes these median portions to be absent and more lateral ones to develop in contact with each other. While this concept can be used to classify sirenoid malformations, its validity as an explanation of the development of the malformations is open to the same criticism as the corresponding theory of cyclopia. It is implicitly based on the assumption of mosaic development, that is, development without correlation of the various parts concerned. That this cannot be taken for granted has been amply demonstrated in cyclopia. The possibility of a primary defect in only one organ in a key position, extending secondarily to other parts by developmental correlations, exists in sirenoid defects as well. Furthermore, the stage used by Feller and Sternberg in which to outline the defects, namely, a 6 mm. human embryo, is too old to use in pointing out the primary lesion, particularly since these authors themselves justly state that in a siren the malformation is determined in the earliest known stages of human development, since the allantois and its vessels are affected.

Very early stages of spontaneous sirens are not known. The youngest human siren on record [275] was a 19 mm. embryo, and its malformation is probably not a typical representative of the group. There is, however, ample information on the genetics, the structure and the embryonic development of related hereditary conditions, namely, taillessness and allied defects in mice [276] and rumplessness in fowl.[277] In all these cases the caudal end of the body is normal until, at a stage characteristic of each form, degeneration sets in and previously normal-appearing parts disintegrate and disappear. In some cases there are associated malformations of the cloaca and the urogenital organs, even though no

274. Klaften, E., and Politzer, G.: Beitr. z. path. Anat. u. z. allg. Path. **99**:70, 1937.

275. Gruenwald, P.: Beitr. z. path. Anat. u. z. allg. Path. **97**:417, 1936.

276. (*a*) Chesley, P., and Dunn, L. C.: Genetics **21**:525, 1936. (*b*) Steiniger, F.: Ztschr. f. menschl. Vererb.- u. Konstitutionslehre **22**:583, 1938. (*c*) Dunn, L. C.; Glueksohn-Schoenheimer, S., and Bryson, V.: J. Hered. **31**:343, 1940. (*d*) Dunn, Glueksohn-Schoenheimer, Curtis and Dunning.[48] (*e*) Glueksohn-Schoenheimer, S.: Genetics **28**:341, 1943. (*f*) Dunn and Glueksohn-Schoenheimer.[54] (*g*) Glueksohn-Schoenheimer, S., and Dunn, L. C.: Anat. Rec. **92**:201, 1945.

277. Landauer, W., and Dunn, L. C.: J. Hered. **16**:153, 1925. Landauer, W.: ibid. **19**:453, 1928. Dunn, L. C., and Landauer, W.: J. Genetics **29**:217, 1935; **33**:401, 1936. Zwilling, E.: Genetics **27**:641, 1942. Landauer, W.: ibid. **30**:403, 1945. Zwilling, E.: J. Exper. Zool. **99**:79, 1945.

degeneration has been found in these parts.   This illustrates once more the inadequacy of an over-all explanation based solely on the extent of the defect in its final form.   In chick embryos another mechanism occurs which leads to defects of the caudal median portions and sometimes to approximation of the lower extremities.   This is a ventral displacement of all axial organs (ourentery) or of the notochord alone (chordentery) with subsequent partial or complete disappearance of the displaced parts.[278]   Severe degrees are probably not viable, and mild degrees may well be the hitherto missing early stages of sporadic rumplessness.   In these embryos the deviation of axial organs creates conditions equivalent to absence of their caudal parts.   The youngest known human siren [275] shows conditions closely resembling these ourenteric chick embryos. Similar defects have been produced experimentally by destroying certain parts of the primitive streak by means of roentgen rays at definite stages.[8c]   According to the current concept of the action of teratogenic factors (see part I) it is easy to understand why the primitive streak with its great morphogenetic activity should be particularly susceptible to damaging influences, such as temporary retardations of metabolism.

In reviewing the subject of sirenoid malformations, Gruenwald [278] comes to the conclusion that some, among them perhaps most of the human sirens, may well develop by a defect of just one leading part with subsequent abnormalities in other parts due to developmental correlations with the primary defect.   This is assumed in analogy with cyclopia. However, in contrast to cyclopia, degeneration of previously normal parts, due to a hereditary deficiency, or dislocation of median organs plays an important role in many other instances.

The common term "interstitial defects" will be used here for all those in which nondefective (though perhaps not entirely normal) regions bound cranially and caudally on a defect of the median organs. Human malformations with defects of the vertebral column and the spinal cord in the lumbar region but not in the sacral portion have been described by Lücke [279] and by Feller and Sternberg.[273]   The latter authors also give references to similar reports in the veterinary literature. According to roentgenograms published by Dobrovolskaia-Zawadskaia and Kobozieff,[280] similar malformations occasionally occur in mice of short-tailed stocks; this indicates a close relationship of interstitial and sirenoid defects.   Wolff [8c] used localized roentgen irradiation to produce in chick embryos interstitial defects with *symptérie,* a median fusion of the upper extremities resembling sirenoid limbs, the more caudal regions not being affected.   Treatment of amphibian embryos with lithium salts may, under properly controlled conditions, result in interstitial defects of

278. Gruenwald, P.: J. Morphol. **81**:97, 1947.

279. Lücke, H. H.: Frankfurt. Ztschr. f. Path. **50**:492, 1937.

280. Dobrovolskaia-Zawadskaia, N., and Kobozieff, N.: Compt. rend. Soc. de biol. **109**:420, 1932.

the notochord.   However, these are not followed by defects of the vertebral column and the spinal cord even though these organs may be retarded or otherwise abnormal.

Complete median defects of the entire length of the body have, to the best of my knowledge, not been reported and probably do not occur.   One malformation of a chick embryo closely approaches this condition.[278]   It shows cyclopia and, throughout the body, a defect of the notochord resembling that seen in cases of chordentery of caudal regions.   The spinal cord is reduced in width throughout, the spinal ganglions are partly median and ventral to the spinal cord, the upper and the lower extremities are abnormally near the midline dorsally, the tail is reduced and the mesonephrons as well as the permanent kidneys are fused in the midline.

SKIN

Much work has been reported on the gross and minute morphologic aspects of prenatally developing abnormalities of the skin and its appendages.   This has been reviewed by Landauer,[281] David,[282] Steiner,[283] Lynch,[221b] Cockayne,[284] Grüneberg [1r] and others.   However, very little is known about the developmental stages of these conditions. Many of the hereditary malformations of the skin, such as hairlessness in certain instances, develop only after birth.

The following conditions have been well studied, though not with regard to their developmental mechanisms: ectodermal dysplasia, affecting in various combinations skin, hair, sweat glands, teeth, eyes and brain [285]; epidermolysis bullosa of hereditary [286] or infectious origin (syphilis, pemphigus,[286a] variola and vaccinia [221b]); hairlessness (hereditary [282] or resulting from maternal dietary deficiency [105]); the Frizzle character of plumage of fowl [287]; congenital tumors of the skin, often multiple [288]; hereditary reduction in the number of mammary glands in guinea pigs [236b] and mice.[289] Several studies are on record of the postnatal

281. Landauer, W.: Ztschr. f. indukt. Abstammungs.- u. Vererbungsl. **42**:113, 1926; **50**:356, 1929.

282. David, L. T.: Ztschr. f. Zellforsch. u. mikr. Anat. **14**:616, 1932.

283. Steiner, K., in Jadassohn, J.: Handbuch der Haut- und Geschlechtskrankheiten, Berlin, Julius Springer, 1932, vol. 4, pt. 1, p. 1.

284. Cockayne, E. A.: Inherited Abnormalities of the Skin and Its Appendages, London, Oxford University Press, 1933.

285. Gordon, W. H., and Jamieson, R. C.: Ann. Int. Med. **5**:358, 1931. Christ, J.: Zentralbl. f. Haut- u. Geschlechtskr. **40**:1,1932. Cole, H. N.; Simmons, J. T., and Stroud, G. M.: J. A. M. A. **129**:723, 1945. Wilkey, W. D., and Stevenson, G. H.: Canad. M. A. J. **53**:226, 1945.

286. (*a*) Herlitz, G.: Acta pædiat. **17**:315, 1935.   (*b*) Davidson, L. T.: Am. J. Dis. Child. **59**:371, 1940.

287. Landauer, W., and Dunn, L. C.: J. Hered. **21**:291, 1930.

288. Wilcox, J. C.: Am. J. Dis. Child. **57**:391, 1939. Vero, F.; Machaček, G. F., and Bartlett, F. H.: J. A. M. A. **129**:728, 1945.

289. Little, C. C., and McDonald, H.: J. Hered. **36**:285, 1945.

influence of defective heat regulation on the entire organism in Frizzle fowl.[290]   Snyder and Doan [291] published an interesting observation on a family with telangiectasia.   The paternal grandmother, the maternal grandfather and both parents of a child had the usual mild type, and the child itself died with a severe, generalized form.   The authors suggest that the gene for telangiectasia may be lethal, being incompatible with life in homozygous form.

Of considerable importance are fetal defects of skin and the ensuing scars, because of their clinical significance as well as their relationship to alleged amniotic adhesions (see the section on extremities).   From case reports, the majority of which do not include histologic observations, it is impossible to judge whether necroses and ulcers have the same nature and cause in all cases.   Liegner [292] describes necrotic lesions of both forearms and indicates that oligohydramnios has caused the parts to press on each other at the sites of these lesions.   Terruhn [293] favors the old amniogenic theory in his discussion of cutaneous defects and scars.   Pantschenko [294], in a report of a case with necroses of both forearms, mentions as possible causes defective hereditary constitution, trophoneurosis and *Lutschflecken* (sucking spots).   Ombrédanne and Lacassie [295] give an excellent discussion of what they call *maladie ulcéreuse intrautérine,* with the conclusion that if there are amniotic adhesions they are the result rather than the cause of ulcers.   These ulcers may be sufficiently deep to cause defects of skeletal parts.   Greig [296] describes median defects of the scalp, in which the normal layers are replaced by a thin membrane.   He considers this membrane as a developmental substitute for the scalp, due to arrested development, rather than a scar.   The median location and the familial occurrence speak against amniotic origin.   Another report of a thin membrane at the vertex, combined with a cutaneous defect of the left leg, has recently appeared.[297]   Ingalls [298] finds bleb formation under the epithelium in the development of these scalp lesions and assumes an inherent abnormality of the structures concerned.   All these reports are concerned with human anomalies.   Hadley [299] describes hereditary defects of the skin of the legs of calves,

290. Benedict, F. G.; Landauer, W., and Fox, E. L.: Bulletin 117, Storrs Agricultural Experiment Station, 1932, p. 130. Landauer, W.: Am. J. M. Sc. **194**:667, 1937; Biol. Symposia **6**:127, 1942.

291. Snyder, L. H., and Doan, C. A.: J. Lab. & Clin. Med. **29**:1211, 1944.

292. Liegner, B.: Monatschr. f. Geburtsh. u. Gynäk. **76**:278, 1927.

293. Terruhn, E.: Arch. f. Gynäk. **140**:428, 1930.

294. Pantschenko, N. A.: Zentralbl. f. Gynäk. **55**:3462, 1931.

295. Ombrédanne, L., and Lacassie: Arch. de méd. d. enf. **33**:199, 1930.

296. Greig, D. M.: Edinburgh M. J. **38**:341, 1931.

297. Callaway, J. L.; Noojin, R. O.; Riley, K. A., and Kuhn, B. H.: J. Pediat. **28**:214, 1946.

298. Ingalls, N. W.: Am. J. Obst. & Gynec. **25**:861, 1933.

299. Hadley, F. B.: J. Hered. **18**:487, 1927.

occurring in certain herds in the ratio of 1 diseased to 3 healthy calves. This clearcut case of a hereditary defect occurring in definite and symmetric locations speaks strongly against amniotic adhesions as the cause. In connection with these considerations Streeter's [76] arguments against amniotic causation of limb defects (the so-called amniotic amputations) should be remembered, as well as the familial and identical forms of these defects in man.[75]

CENTRAL NERVOUS SYSTEM

The commonest gross malformations of the central nervous system are dysraphia (clefts dorsally in the midline: encephaloschisis and myeloschisis) and hydrocephalus. It has been commonly assumed that defects of the former type are due to failure of the neural groove to close. This is doubtless true for the majority of cases. However, Bonnevie [300] showed that in two different hereditary malformations of mice the previously closed brain breaks open again, thus producing a condition which may later on be indistinguishable from primary failure to close. In one of these instances (the "shakershort" mouse) abnormal brain development causes abnormalities of the inner ear although the otic vesicles are normal in early stages. Kaven [229b] observed changes essentially like those described by Bonnevie, in mouse embryos irradiated with roentgen rays in utero. Perhaps a comparable mechanism of secondary rupture can explain the observation of Paff [110] that chick embryos may show dysraphia when treated with colchicine at the age of 48 hours, that is, after the neural tube should have closed completely. Another instance in which the simple explanation—failure of a normal neural plate to close—may not be applicable is spina bifida of the caudal portions of the body. There is in the embryo a region in which the neural tube forms not by closure of a groove but by hollowing out of a solid cord; this occurs in the trunk-tail node. Just how large the caudal part of the body is which develops in this manner is not known. A region where development almost certainly occurs from the trunk-tail node is the base of the tail, where typical myeloschisis has been observed in a sheep embryo.[301] The appearance of the abnormality is too regular to make one assume a secondary dehiscence, and the most probable explanation is that a neural plate was formed where it would not normally develop. This consideration may very well hold for the sacral region of the human embryo, where dysraphia is relatively common. This will not be definitely known until the exact extent of body formation from the trunk-tail node in man is determined. The clinical aspects of dysraphia have been discussed by several authors.[302]

---

300. Bonnevie, K.: Genetica **18**:105, 1936; Skr. Norske Vid. Ak. Oslo **9**:39, 1936.

301. Gruenwald, P.: Unpublished data.

302. Ingraham, F. D., and Swan, H.: New England J. Med. **228**:559, 1943.

A peculiar and teratologically interesting form of encephaloschisis and myeloschisis occurs frequently in chick embryos but has, to the best of my knowledge, not been seen in man and mammals.   This is platyneuria, characterized by a flat, abnormally thick neural plate. If there is any attempt to form a neural groove, it is not by rising of the borders as this occurs in early stages of normal development or remains permanently in some cases of the usual dysraphia but by a narrow, steep infolding of the median portion alone while the lateral parts remain flat.[84] The differentiation in later stages is severely disturbed in all cases, a phenomenon which is not observed in the usual dysraphia.   In the brain the usual divisions cannot be recognized, and the eyes are either absent or so severely deformed that one may not recognize them until in late stages by their pigmentation. Histogenesis is also abnormal, and numerous rosettes may be found in the brain substance.[303] Thus platy-neuria is not only an inhibition of the normal folding and closure of the neural plate but a thorough disturbance of many phases of its organiza-tion as well.   In many laboratories platyneuria occurs frequently, per-haps owing to unfavorable conditions of incubation, such as inadequate ventilation. Experimentally, platyneuria has been produced by a great increase of the concentration of the carbon dioxide in the air.[201] In this connection a recent report of Patten [304] is of great interest. It indicates that in human dysraphia there is also an abnormal growth tendency, producing an abnormally large neural plate. This is considered as a possible cause of the failure to close. I have confirmed this in part of the cases at my disposition. The thickening of the neural plate is not accompanied by the other severe disturbances found in platyneuria of the chick.

It seems that there are rare cases in which mechanical injury causes the brain of the human embryo to rupture at points distant from the normal line of closure. These cases have added interest because there may be nodes of nervous tissue growing within the lungs, owing probably to embolism at the time of the injury.[81]

Hydrocephalus is usually the result of obstruction of the route which the cerebrospinal fluid takes from the ventricles to the subarachnoid space: At least three different kinds of hereditary hydrocephalus of mice are known, and these have been studied in some detail. In one of them the aqueduct was obliterated.[305] In another one an abnormal con-figuration of the roof of the fourth ventricle is caused by a disturbance of the growth of the cartilaginous base of the skull.[232a] The third muta-

303. (a) Podmaniczky, T.: Frankfurt. Ztschr. f. Path. 5:255, 1910. (b) Gruen-wald, P.: Anat. Rec. 94:518, 1946.

304. Patten, B. M.: Anat. Rec. 94:487, 1946.

305. Clark, F. H.: Anat. Rec. 58:225, 1934.

tion,[306] as well as hydrocephalus produced in embryos by roentgen rays,[307] has not been investigated embryologically.

Lichtenstein [308] has given a plausible explanation of those relatively numerous cases in which hydrocephalus of man is associated with spina bifida of the lower portion of the spinal cord. He found that relative shortening of the spinal cord, which normally results in a cranial movement of the lower segments, produces a caudad movement of the upper segments if the lower portion is held in place. This draws parts of the brain stem and cerebellum through the foramen magnum into the spinal canal (Arnold Chiari malformation) and thus interferes with the drainage of fluid from the ventricles. Ingraham and Scott [309] studied 20 cases of this malformation in detail and observed, in addition to other phenomena, that all had microgyria. I have made the same observation in a smaller series of cases. Certain forms of hydrocephalus have been *treated surgically*.[310]

Porencephaly is thought to be due either to degeneration of previously normal brain tissue—for example, by occlusion of blood vessels—or to rupture of the brain in hydrocephalus.[311] Yakovlev and Wadsworth [312] have shown that there are, in addition to porencephaly due to secondary changes, rare conditions in which a developmental anomaly must be assumed (schizencephaly). These defects are strictly symmetric and in typical locations.

An accidental discovery in mice of normal behavior was a hereditary defect of the corpus callosum.[313]

Several abnormalities of the minute structure of the central nervous system, often associated with gross malformations, are on record. Rosette formation has been suggested as a possible link between malformations and neoplasms of the nervous tissue, as it is observed in both conditions.[303] When they appear as a malformation, rosettes consist of minute cavities with lining cells in a radial arrangement, resembling ependyma. Numerous rosettes are often found in the brains of platyneuric chick embryos.[303] Occasionally rosettes occur associated with other malformations. Their development has not been adequately followed as yet. Their form and location, as well as their similarity to

306. Grüneberg, H.: J. Genetics **45**:22, 1943.

307. Job, Liebold and Fitzmaurice.[91] Kaven.[92]

308. Lichtenstein, B. W.: Arch. Neurol. & Psychiat. **47**:195, 1942.

309. Ingraham, F. D., and Scott, H. W.: New England J. Med. **229**:108, 1943.

310. Sachs, E.: J. Mt. Sinai Hosp. **9**:767, 1942. Michelsen, J. J.: Am. J. Ment. Deficiency **48**:15, 1943.

311. Marburg, O.; Rezek, P. R., and Marks, M. B.: J. Neuropath. & Exper. Neurol. **4**:43, 1945.

312. Yakovlev, P. I., and Wadsworth, R. C.: J. Neuropath. & Exper. Neurol. **5**:116 and 169, 1946.

313. King, L. S.: J. Comp. Neurol. **64**:337, 1936.

rosettes of the retina, which are known to develop independent of the ventricle,[314] suggest that they also develop in loco and are at no time continuous with the ventricles. Occasionally rosettes are found in the brains of embryos with hereditary microphthalmia and rosettes of the retina.[314] Groups of rosettes occur normally in the human brain—for example, in the medulla oblongata near the insertion of the tela choroidea.

Other kinds of cavities and rarefactions in the brain tissue occur normally in embryos of birds and mammals, including man, and disappear without leaving a trace.[315] The only indication of a teratologic significance of these formations is the observation that they also occur in association with gross malformations of the nervous system (brain, spinal cord, cranial and spinal ganglions) in regions where they are not normally found.[315]

There are several syndromes of multiple abnormal growths of the central nervous system and other organs, and perhaps the best studied of these is tuberous sclerosis. There is fair agreement that the condition is congenital, even though in the reported cases the patients were not very young persons. There are multiple tissue malformations involving the brain, the eyes, the heart, the kidneys and the skin; they show a tendency to form noncancerous and, less frequently, cancerous tumors. Feriz[316] suggests that the abnormal foci develop as overgrowth of atypical, functionally inferior tissue, at the points of defects due to inhibition of the genesis of the normal tissue; this explains the coexistence of defective and excessive formations. Yakovlev and Guthrie[317] classify tuberous sclerosis as one of several types of congenital ectodermoses, and they emphasize the existence of abortive forms which on superficial examination may pass for idiopathic epilepsy. Moolten[318] describes tuberous sclerosis as an example of "disseminated hamartiosis" and explains it in vague terms as due to "a defective mechanism of induction by embryonic organizers." It was shown in foregoing paragraphs that there are many mechanisms of development of simple and combined malformations which Moolten does not consider and rule out. Obviously, all theories based on the final structure of the malformation alone are at best working hypotheses until they are confirmed either by examination of early stages or by experimental reproduction of the condition.

Nonhereditary feeblemindedness is due in a large proportion of the cases to causes operating during embryonic development or at birth. Research has shown or suggested several possible causes during the

314. Gruenwald, P.: Anat. Rec. **88**:67, 1944.

315. Gruenwald, P.: J. Neuropath. & Exper. Neurol. **4**:178, 1945.

316. Feriz, H.: Virchows Arch. f. path. Anat. **278**:690, 1930.

317. Yakovlev, P. I., and Guthrie, R. H.: Arch. Neurol. & Psychiat. **26**:1145, 1931. Yakovlev, P. I.: ibid. **41**:119, 1939.

318. Moolten, S. E.: Arch. Int. Med. **69**:589, 1942.

past few years. Besides those causes which have been known for a long time, one has to consider: syphilis, rubella [217] or toxoplasma infection [319]; maternal endocrine deficiency [144] or abnormal conditions of the endometrium [320] in the case of mongolism; maternal dietary iodine deficiency [321]; injury caused by mechanical trauma or anoxia at birth [208]; heterospecific pregnancy (Rh blood group and less frequently others) [322]; roentgen irradiation of the fetus (see part I). Now that these causes of feeblemindedness begin to be appreciated, a wide field is opening up for preventive medicine. [323] In addition, some of the just mentioned causes also confine persons to institutions by producing deafmutism or blindness. In the cases of syphilis, toxoplasmosis, roentgen irradiation and birth injury the morphologic basis of the disturbance is established with relative ease; in the cases of anomaly associated with rubella occurring during pregnancy and of mongolism the mechanism is unknown. For the damage that occurs in heterospecific pregnancy several mechanisms have been suggested. Damage of the brain may result from damage of the liver [322d] or from a disturbance of development due to anemia and anoxia.[322c] Recently Wiener and Brody [324] observed thrombi in cerebral vessels. The well known condition of kernicterus (encephalomyopathy with icterus) indicates damage of the brain as it is not explained by jaundice alone.[322a,d] However, it is not yet established whether in all infants with kernicterus disturbances develop if they survive, nor do investigators know whether mental deficiency is limited to patients who had kernicterus, since the presence of the latter cannot be determined in the living patient. A more comprehensive discussion of the effects of heterospecific pregnancy will be given in a later section (page 557).

There are several types of hereditary defects associated with mental deficiency, which are only recently being identified and segregated from one another and from those without a genetic basis.[325]

319. Cowen, D.; Wolf, A., and Paige, B. H.: Arch. Neurol. & Psychiat. 48:689, 1942. Zuelzer.[223d]

320. Mayerhofer, E.: Ann. pædiat. 154:57, 1940. Engler, M.: Am. J. Ment. Deficiency 50:27, 1945.

321. Stoot, H., and Gupta, S. P.: Indian J. M. Research 21:655, 1934. Benda.[146]

322. (a) Zimmermann, H. M., and Yannet, H.: Am. J. Dis. Child. 49:418, 1935. (b) Yannet, H., and Lieberman, R.: Am. J. Ment. Deficiency 49:133, 1944; 50:242, 1946; J. A. M. A. 130:335, 1946. (c) Cook, R.: J. Hered. 35:133, 1944. (d) Gilmour, J. R.: Arch. Dis. Childhood 19:1, 1944. (e) Snyder, L. H.; Schonfeld, M. D., and Offerman, E. M.: J. Hered. 36:9, 334, 1945. (f) Doctor, J. M.: J. Pediat. 27:327, 1945.

323. Gruenwald, P.: Am. J. M. Sc., to be published.

324. Wiener, A. S., and Brody, M.: Science 103:570, 1946.

325. Allan, W.; Herndorn, C. N., and Dudley, F. C.: Am. J. Ment. Deficiency 48:325, 1944. Benda, C. E.: ibid. 49:32, 1944. Halperin, S. L.: ibid. 50:8, 1945. Snyder.[45b]

Correlations between various parts of the body and the region of the central nervous system which supplies them have been observed in malformations. Tsang[326] and Baumann and Landauer[327] found increased numbers of cells in the ventral horns of the spinal cord corresponding to polydactylous limbs in mice and chicks, respectively. In experimental chick embryos the differentiation of the spinal cord is influenced by extirpation or transplantation of the primordia of limbs.[328] Chase[329] describes in detail the defective development of certain cranial nerves and brain centers in mice with hereditary microphthalmia. Conversely, defective innervation may affect the development of various organs. The differentiation of the skeleton in nerveless transplanted limbs of chick embryos has been examined by Hamburger and Waugh.[330] The occurrence of clubfeet in newborn infants with lumbosacral spina bifida is well known.

<div align="center">EYES</div>

Very large numbers of ocular malformations, hereditary as well as sporadic, are on record. Mann's book and Howe's[331] enumeration of hereditary malformations of the eye show the number and the variety of types. Stockard[1a] has expressed what is probably the generally accepted explanation of this great tendency of the eyes to abnormal development. He assumes that the rapid rate of growth and development in early stages accounts for the high susceptibility to various injurious agents which act by retarding development at a given moment. There is no known type of teratogenic agent which will not under certain conditions produce ocular malformations (see part I).

The present discussion will be limited to a few instructive examples of ocular malformations which have been thoroughly investigated as to their embryonic development.

Many malformations of the eye have been described as microphthalmia because they are associated with a reduction in the size of the eye. Often microphthalmia has erroneously been called anophthalmia if the small eye was not externally visible. True anophthalmia is very rare, and when it occurs it may be due to regression rather than to complete primary absence of the primordium of the eye.[332] Best studied are several hereditary forms of microphthalmia. While in each of these

326. Tsang, Y. C.: J. Comp. Neurol. 70:1, 1939.

327. Baumann, L., and Landauer, W.: J. Comp. Neurol. 79:153, 1943.

328. Bueker, E. D.: J. Exper. Zool. 93:99, 1943.

329. Chase, H. B.: J. Comp. Neurol. 83:121, 1945.

330. Hamburger, V., and Waugh, M.: Physiol. Zoöl. 13:367, 1940.

331. Howe, L.: A Bibliography of Hereditary Eye Defects, Eugenics Record Office Bulletin 21, Cold Spring Harbor, Carnegie Institution of Washington, 1928.

332. Chase, H. B., and Chase, E. B.: J. Morphol. 68:279, 1941.

the changes in embryos are fairly uniform and follow a definite pattern, no uniformity of structure or development is seen when the malformations caused by different mutations are compared. In the following discussion microphthalmia will be divided into two types comprising, respectively, severe general underdevelopment of all derivatives of the optic cup, and abnormalities restricted primarily to one part—for example, the choroid fissure or the retina.

Representative of hereditary microphthalmia with severe general inhibition of development are the mutations described by Chase and Chase [332] and by Browman and Ramsey.[333] The former authors examined the genetics and the embryologic aspects of a mutation causing anophthalmia or microphthalmia in the mouse. The optic vesicles, and often the cups, form as in normal embryos. However, the choroid fissure never closes, remaining as a coloboma. Then retardation sets in, and in severe cases degeneration seems to occur, so that at birth all remnants of the eye may have disappeared or may be too inconspicuous to be found. A lens vesicle forms only if the optic vesicle comes close to the epidermis.

Browman and Ramsey examined the embryonic development of microphthalmia in a strain of rats and found that an optic cup always exists in the embryo. In later stages it fails to grow and differentiate normally, and at birth the small rudiment of an eye may be degenerating. Lenses are formed in these eyes. The authors attribute much importance to failure of the hyaloid artery to develop, so that vessels of the rim of the optic cup are the only ones to supply the interior. Development goes on normally only as long as the tissues do not depend on the blood supply of the hyaloid artery. The microphthalmic eyes as well as many otherwise normal eyes in animals of this strain are devoid of an optic nerve. In both of the mutations which have thus far been described, not only growth but also histogenesis of the eye is severely impaired.

Sporadic microphthalmia is relatively common and often reaches degrees comparable with the just described hereditary forms. Here the embryologic aspects cannot be investigated systematically, and it is necessary to examine whatever forms and stages happen to present themselves, often without knowing what the earlier or later phases of the same malformation may be. This has been done in a fairly large number of chick embryos,[15] and the following conclusions resulted from the interpretation of the findings. Malformations grossly visible as microphthalmia are often not limited to the reduced eye. The adjacent parts of the forebrain, as well as the normal-sized other eye may be affected as well. The ocular malformations do not conform to any one type. Any part except the pigmented epithelium may be com-

333. Browman, L. G., and Ramsey, F.: Arch. Ophth. 30:338, 1943.

pletely absent. The only manner in which these varied defects can be accounted for is that of an abnormal determination of the parts in the early primordium of the eye. An indication of the stage in which this determination can still be altered is given by the predominance of this kind of microphthalmia on the left side. This is most probably due to a temporary handicap of the left eye at the time when the head turns to the side, in embryos of about 2 days. The left eye is then far from the egg shell and does not receive as much oxygen by diffusion as the right eye, which is just under the shell. Circulatory oxygen supply sets in somewhat later, and during this interval a high percentage of normal embryos show a temporary lag in the development of the left eye. This is the only known factor which may selectively damage the left eye and thus increase its susceptibility to other injurious agents. At that time the optic vesicle begins to transform itself into the cup, and at this late stage the determination of the various parts of the eye— is apparently not sufficiently rigid to prevent changes.

The interpretation of these malformations suggested that teratogenic agents of the following three kinds are all active: An agent within the eye itself is its high susceptibility due to rapid development (see opening paragraph of this section). An agent extrinsic to the eye but within the embryo is the rotation of the head which favors malformations of the left eye. That these two agents in themselves are not sufficient is obvious, since they are present in the normal embryo. However, they influence the intensity and the localization of the reaction to the third kind of factor, apparently extrinsic to the embryo. A hint of the possible nature of the latter is given by Stockard,[70] who found an increased incidence of sporadic microphthalmia in eggs incubated in an atmosphere contaminated by laboratory fumes. This may well hold for the cases used in the investigation just quoted. Landauer[1J] emphasizes that a higher percentage of maldeveloped chicks are found in eggs incubated under unfavorable conditions. A higher incidence of microphthalmia of the left side of the chick embryo was also found elsewhere in sporadic cases,[204b] as well as in selenium-induced conditions[49] and in homozygous Creeper embryos.[46] The last-mentioned observation led Cairns to refer for the first time to the unequal oxygen supply of the eye when the head rotates; in that particular case the condition is aggravated by an inadequate circulation in later stages.

Detailed information is available on a type of microphthalmia of mice which is genetic so far as the causative agent develops on the basis of a hereditary abnormality; the immediate action on the eye is mechanical if that agent, a bleb of cerebrospinal fluid moving under the epidermis, happens to reach the region of the eye.[334] The

---

334. Bonnevie, K.: J. Exper. Zool. 67:443, 1934.

hereditary condition is usually referred to as "myelencephalic blebs"; it will be described in a later section (page 556) in its various manifestations.

A number of mutations are known which produce milder forms of microphthalmia with little impairment of histogenesis and no secondary degeneration of the eyes, but with formation of a coloboma. All colobomas to be considered here are clefts of the eyeball produced by abnormalities of the choroid fissure. Diagrams of the form and the development of the three fundamental types of coloboma are given by Mann [11] and Gayer.[203] One type is the embryonic coloboma in which the fissure remains open. The boundary of the retina and the pigmented epithelium is at the lips of the fissure. The second type, embryonic coloboma with orbital cysts, also has an open fissure, but the retina extends beyond the lips and forms both layers of the cup in an area adjacent to the fissure. The outer layer is inverted, with the inner limiting membrane facing outward. These inverted portions of retina in the outer layer of the cup near the fissure tend to bulge outward in later stages, forming orbital cysts. The third type is the ectatic coloboma. This has in common with the second type the presence of inverted retina in the outer layer near the fissure, but the fissure closes later on. As in all areas where the outer layer consists of retina instead of pigmented epithelium, the connective tissue coats of the eyeball are deficient, and both layers of the cup bulge outward together.

Von Hippel [335] was the first to examine systematically the embryonic stages of hereditary coloboma in rabbits. Other authors [336] have since carried out similar investigations in apparently unrelated strains of rabbits with the same malformation. All agree that the choroid fissure remains open and that an increased amount of mesenchyme is found between its lips and in the interior of the cup. Hippel assumes that this mesenchyme causes coloboma by preventing closure of the fissure, whereas von Szily maintains that the fissure, that is, the ectodermal tissue, is primarily abnormal and that the mesenchyme enters because of this abnormality. All authors found inverted retina in the outer layer of the optic cup adjacent to the fissure, and all three of the aforementioned types of coloboma may result. Von Szily has published illustrations of numerous plastic reconstructions of these eyes. Warkany and Schraffenberger [164] have observed the development of coloboma in the embryos of rats with vitamin A deficiency.

Much experimental work has been done with chick embryos homozygous for the Creeper factor, in which coloboma develops if they

---

335. von Hippel, E.: Arch. f. Ophth. **55**:507, 1903.

336. Seefelder, R.: Verhandl. d. deutsch. ophth. Gesellsch. **42**:210, 1920. Koyanagi, Y.: Arch. f. Ophth. **104**:1, 1921. von Szily.[1c]

reach sufficiently late stages.   If incubated without experimental interference, the majority of the homozygous Creeper embryos die on the fourth day, apparently because of a defective circulation of blood in the wall of the yolk sac.[46] Those which survive have phokomelia and coloboma and die shortly after hatching time.   The various manifestations of the Creeper mutation will be summarized later in this review (page 554). Gayer [203] found that in eyes of homozygous Creeper embryos transplanted to the flanks of normal embryos colobomas develop similar to those of phokomelic embryos.   However, eyes of normal control embryos transplanted in the same manner show colobomas of the same type.   Thus the altered environment produces a phenocopy of the Creeper coloboma in genetically normal eyes.   Orthotopic transplantation of a homozygous Creeper eye to the ocular region of a normal embryo produces an eye without coloboma.[337] The reverse experiment, namely, transplantation of a normal eye to the corresponding site in a homozygous Creeper host, was successful only in 1 case, owing to the high mortality of the Creeper hosts.   The transplanted eye had a coloboma.   All these experiments demonstrate the importance of an influence of the environment on the optic cup in the formation of coloboma.   Gayer and Hamburger [337] conclude that in the homozygous Creeper embryo the optic cup is primarily normal and that only its environment is abnormal.   Landauer [338] points out that this is only one of several possible interpretations of the experiments and that the normal properties of the cup are not proved, even though the influence of the environment on the cup is beyond doubt.

In addition to cases of microphthalmia with a fairly good differentiation of most parts and with coloboma, there are other small eyes in which the principal parts are also differentiated but the retina shows histologic abnormalities.   Rosettes and folds of the retina have been found under various conditions.   They occur in a strain of chickens with hereditary bilateral microphthalmia caused by a simple recessive gene.[339] The embryologic aspects of this condition have been studied,[314] and it has been shown that the rosettes arise in previously normal eyes without any folding and that their cavities are at all times separated from the cleft between the two layers of the cup.   Later on folds develop in such a manner that the rosettes are on their crests, or else they are independent of rosettes.   As soon as these abnormal differentiations appear, the eyes lag in growth.

Similar rosettes have repeatedly been found in the eyes of mammalian,[89] including human,[90] embryos exposed in utero to roentgen

337. Gayer, K., and Hamburger, V.: J. Exper. Zool. **93**:147, 1943.
338. Landauer, W.: Am. Naturalist **78**:280, 1944.
339. Jeffrey, F. P.: J. Hered. **32**:310, 1941.

rays.· They also occur, though rarely, without apparent cause.[340] Goldstein and Wexler [90c] hold that rosettes may be formed from retinal folds as well as from dislocated cells, the latter apparently because the normal cells in the same layer do not normally multiply as do those of the rosettes. While it is true that the cells of the rosettes resemble those bordering on the cleft between the .two walls of the optic cup, there is little to be gained by referring to them as dislocated, since all cells of the retina are derived from that one layer. In the rosettes the cells have apparently retained the ability to multiply, which is normally lost when cells move away from the outer border, and have at the same time failed to differentiate like the surrounding cells.

An entirely different abnormality of the retina, occurring in eyes of normal size, has been found as a mutation in mice. The formation of rod cells is partly or completely inhibited, depending on the presence of modifying genes.[341]

The optic nerve may be absent in cyclopic, in microphthalmic or in otherwise normal eyes. Fischel [5] assumes that cyclopic eyes are devoid of the nerve because the cyclopic defect eliminates the optic stalk, the eye subsequently constricting itself off from the brain. Adelmann [267d] describes this process in lithium-induced cyclopia. However, in part of the cases of cyclopia and in cases of sporadic microphthalmia of chickens absence of the optic nerve has a different cause.[265] All these malformed eyes have optic stalks in early stages. If no nerve fibers grow into the stalk either because there is no retina or because the usual pathway is obstructed by absence of the choroid fissure, the stalk degenerates at the end of the first week of incubation and soon disappears completely. In several cyclopic eyes without optic nerves indications of a primary absence of the choroid fissure were found.[265] In a strain of microphthalmic rats described by Bowman and Ramsey [333] (see page 518) the optic nerve is frequently absent., The authors consider absence of the hyaloid artery as the cause, without explaining the mechanism. Atrophy of the previously normal optic nerve has been found 'in cattle as a sequel of abnormal narrowness of the bony canal. This, as well as paralyses, appeared in the offspring of cows with a nutritional deficiency of an unidentified factor, perhaps vitamin A.[342]

The lens may be affected during embryonic development by a variety of factors. The action of chlorobutanol on the early phases of its formation in amphibians has been examined by Lehmann.[108] This compound produces undersized but otherwise normal lenses. In later stages of the

340. Jaensch, R. A.: Arch. f. Ophth. **116**:464, 1925.

341. Keeler, C. E.: J. Exper. Zool. **46**:355, 1927.

342. Moore, L. A.; Huffman, C. F., and Duncan, C. W.: J. Nutrition **9**:533, 1935.

development of the eye, cataract may develop on a hereditary basis [343] or through the action of chemicals (galactose,[131] naphthalene [35]) or roentgen rays.[344]

Numerous reports have recently described cataract in children of mothers who contracted rubella during the early months of pregnancy.[345] The mechanism is not known.

The development of accessory structures of the eye, such as extrinsic muscles, cornea, lids, conjunctiva and lacrimal glands, is apparently independent of the eyeball to a considerable degree, and, as a result, these structures are often better developed than the eye itself in cases of microphthalmia.[346]

DIGESTIVE AND RESPIRATORY TRACTS

Abnormalities of the teeth will be treated in part III of this review, since most of the experimental work has been done with continuously growing teeth of adult animals.

Major malformations of the digestive tract have gained clinical importance during the past few years since technics have been perfected for the surgical treatment of the most common forms.[347] Accounts of the development of malformations, such as atresia, are based largely on observations of the final conditions and on conjectures concerning the early stages. It has, for instance, been claimed that atresia of the duodenum is favored by the epithelial occlusion of the lumen which normally occurs in the embryo, the lumen in abnormal cases not being properly canalized but penetrated by connective tissue. This, however, does not explain the very similar atresia occurring elsewhere in the intestine, where there is no temporary epithelial occlusion. In some of these cases an intestinal loop may have been cut off when it failed to retract properly from the normal umbilical hernia.[348] Atresia of the esophagus combined with tracheoesophageal fistula in its most common form, in which the lower segment of the esophagus opens into the trachea, has received much consideration. The hypothesis that it is due to delay of the process whereby the respiratory anlage is separated from the digestive tube and to a consecutive process whereby part of the esophagus is incorporated into the posterior wall of the trachea is supported by the fact that esophageal tissue has been seen in the posterior wall of the trachea in newborn infants and, in an early stage, in a chick embryo.[349]

343. Gregory, P. W.; Mead, S. W., and Regan, W. M.: J. Hered. **34**:124, 1943. Lutman, F. C., and Neel, J. V.: Arch. Ophth. **33**:341, 1945.

344. von Hippel.[88] Kaven.[92]

345. Footnotes 217 and 218.

346. Gruenwald.[1s] Chase and Chase.[332]

347. Swenson, O., and Ladd, W. E.: New England J. Med. **233**:660, 1945.

348. Misgeld, G. C.: Virchows Arch. f. path. Anat. **310**:697, 1943.

349. Gruenwald, P.: Anat. Rec. **78**:293, 1940; **85** (supp.):23, 1943.

Malformations of the liver and the pancreas consist largely of occlusion of their ducts at various points and of cysts of the ducts. Polycystic malformations of the liver, the pancreas and the kidneys or of two of these organs are relatively frequently combined in one person.[350] This is intriguing since the development of the pancreatic and hepatic ducts differs considerably from that of the renal tubules.

Cysts, atresia and stenosis of bile ducts are multiple in a high proportion of cases.[351] No direct observations of developmental stages of these malformations exists. Numerous speculations concerning this subject will not be reviewed. Moolten [350d] has recently favored the hypothesis that polycystic disease of the liver and the kidneys is a type of hamartiosis, that is, a multiple tissue malformation, and that it is due to deficient organizer action. Atresia of ducts, on the other hand, is thought to be aplasia due to a deficiency of the tissue itself. However, it is obvious that atresia of the extrahepatic bile ducts, as in Moolten's own case, cannot be aplasia. The duct must have been present at one time, or no liver could have been formed. If atresia is due to a defect of tissue, this defect cannot be in the form of aplasia but must occur only as a secondary change.

Cystic fibrosis of the pancreas is of considerable clinical importance because its signs must be differentiated from those of other diseases not caused by malformations. At birth the changes in the pancreas are in their early phases. There is moderate distention of small ducts with inspissated secretion, as well as slight fibrosis. These changes progress to severe fibrosis with destruction of many acini and cystic dilatation of ducts. The deficiency of pancreatic secretion in the intestine causes either meconium ileus in the fetus or a severe nutritional deficiency and disturbance of intestinal function in the infant. Meconium ileus is due to inadequate digestion and subsequent hardening of the meconium.[352] This meconium can easily be digested by pancreatic enzymes in the test tube, but attempts to dissolve it by the same agents in vivo have not yet been successful.[353]

Four explanations have been offered for the cause and development of cystic fibrosis of the pancreas. · In evaluating these, one must remember that any obstruction of the flow of secretion may produce cystic fibrosis and that it is not necessarily due in all cases to the same cause.

350. (*a*) Moschcowitz, E.: Am. J. M. Sc. **131**:674, 1906. (*b*) von Meyenburg, H.: Beitr. z. path. Anat. u. z. allg. Path. **64**:477, 1918. (*c*) Rumler, E.: Virchows Arch. f. path. Anat. **292**:151, 1934. (*d*) Moolten, S. E.: New York State J. Med. **43**:727, 1943.

351. Feyrter, F.: Virchows Arch. f. path. Anat. **271**:20, 1929.

352. Kornblith, B. A., and Otani, S.: Am. J. Path. **5**:249, 1929. Hurwitt, E. S., and Arnheim, E. E.: Am. J. Dis. Child. **64**:443, 1942.

353. Farber, S.: J. Pediat. **24**:387, 1944.

Several workers [354] have proved by an examination of serial sections of the pancreas that congenital stenosis or atresia of the main duct was present in their cases. In other instances of pancreatic fibrosis no anatomic malformation of this kind was found in serial sections.[355]

Andersen [356] found in a considerable percentage of cases of pancreatic fibrosis squamous metaplasia of the ducts, and she suggested that this may be the cause of stenosis and of retention of secretion. The interpretation of this metaplasia is difficult because it may also be the sequel of vitamin A deficiency secondary to pancreatic achylia. This is demonstrated by a case examined by Oppenheimer,[354a] in which there was squamous metaplasia of the bronchi and salivary ducts, while atresia of the pancreatic duct accounted for pancreatic fibrosis and vitamin A deficiency.

Brody [221c] found in cases of pancreatic fibrosis inclusion bodies suggesting a fetal infection. However, occlusion bodies occur not rarely in infants without pancreatic disease and, on the other hand, are absent in the majority with that disease. That they are related to the cause of pancreatic fibrosis is therefore doubtful.

Farber [357] assumes that an abnormal secretion is produced which is so viscid that it occludes the ducts ("mucoviscidosis"). This is thought to be caused by abnormal stimulation originating from the autonomic nervous system. Farber [358] produced in kittens with pilocarpine a condition similar in all important respects to pancreatic fibrosis of human infants. More recently Glanzmann [359] and Riniker [360] have also referred to an abnormally thick secretion, and Andersen and co-workers [361] have voiced an opinion similar to that of Farber. The associated pulmonary changes (bronchiectasis, emphysema) are explained by Farber [357] on the basis of viscid bronchial secretion, whereas Andersen and co-workers [362] assume a secondary vitamin A deficiency with squamous metaplasia of the bronchial epithelium.

The familial occurrence of pancreatic fibrosis has long been known. It has recently been discussed by Andersen and Hodges.[361b]

The subject of pancreatic fibrosis has recently been reviewed by Wiglesworth.[363]

354. (*a*) Oppenheimer, E. H.: Arch Path. **29**:790, 1940. (*b*) Footnote 352.

355. Baggenstoss, A. H., and Kennedy, R. L., Jr.: Am. J. Clin. Path. **15**:64, 1945.

356. Andersen, D. H.: Am. J. Dis. Child. **56**:344, 1938; J. Pediat. **15**:763, 1939.

357. Farber, S.: Arch. Path. **37**:238, 1944; J. Michigan M. Soc. **44**:587, 1945.

358. Farber, S.: Am. J. Dis. Child. **64**:953, 1942.

359. Glanzmann, E.: Ann. pædiat. **166**:289, 1946.

360. Riniker, P.: Ann. pædiat. **166**:314, 1946.

361. (*a*) Di Sant'Agnese, P. E., and Andersen, D. H.: Am. J. Dis. Child. **72**:17, 1946. (*b*) Andersen, D. H., and Hodges, R. G.: ibid. **72**:62, 1946.

362. Footnotes 356 and 361.

363. Wiglesworth, F. W.: Am. J. M. Sc. **212**:351, 1946.

Large numbers of newborn infants die with poorly aerated lungs. In some cases this is doubtless due to extrapulmonary factors, such as disturbances of the respiratory center or mechanical obstruction of the air passages. In many others, however, the cause is probably in the lungs themselves.[364] This problem is closely linked with that of fetal respiratory movements. It has been claimed that the contents of the amniotic sac may be aspirated in utero and that this may cause an inflammatory reaction of the lung tissue which later on prevents expansion of the alveoli.[207b] Some authors hold that respiratory movements, by which amniotic fluid is necessarily aspirated, regularly occur in the fetus and are even instrumental in the normal development of the lungs.[365] The latter contention has been disproved by Potter and Bohlender,[366] who showed in a case of complete atresia of the larynx and in one of accessory lung that lung tissue which could not have expanded during respiratory movement nevertheless developed normally. The assumption that normal fetal lungs are partially expanded [207c] is based on inconclusive evidence. A slight experimental interference, such as anesthesia and laparotomy, even without opening the uterus, may cause anoxia of the fetus, particularly near term. If one sees respiratory movements under these conditions, this does not necessarily represent the normal condition.

It is true that almost all stillborn infants have expanded alveoli filled with amniotic fluid,[367] but this is not the normal state. Windle [200b] has shown that if the trachea of the animal fetus is clamped before it is in anoxia, the lungs are atelectatic. If a little more time elapses during the experiment, the fetus begins to gasp in utero and then shows histologically the same condition which prevails in the stillborn infant. It is common experience that large parts of the lungs of the young infant with poor respiration have collapsed alveoli. Therefore several authors [368] assume that there are normally no intrauterine respiratory movements. If such movements occur during temporary anoxia of the

364. Farber, S., and Wilson, J. L.: Am. J. Dis. Child. **46**:572, 1933. Wilson, J. L., and Farber, S.: ibid. **46**:590, 1933.

365. Snyder, F. F., and Rosenfeld, M.: Proc. Soc. Exper. Biol. & Med. **36**:45, 1937; J. A. M. A. **108**:1946, 1937. Bonar, B. E.; Blumenfeld, C. M., and Fenning, C.: Am. J. Dis. Child. **55**:1, 1938. Patterson, J. C., and Farr, J. T.: Canad. M. A. J. **41**:31, 1939. Snyder, F. F.: Am. J. Obst. & Gynec. **41**:224, 1941. Davis, M. E., and Potter, E. L.: J. A. M. A. **131**:1194, 1946.

366. Potter, E. L., and Bohlender, G. P.: Am. J. Obst. & Gynec. **42**:14, 1941.

367. (*a*) Windle.[206] Potter, E. L., in discussion on Zettelman.[369d] (*b*) Gruenwald, P.: Am. J. Obst. & Gynec. **53**:996, 1947.

368. (*a*) Farber and Sweet.[207a] (*b*) Windle, W. F.; Becker, R. F.; Barth, E. E., and Shulz, M. D.: Surg., Gynec. & Obst. **69**:705, 1939. (*c*) Whitehead, W. H.; Windle, W. F., and Becker, R. F.: Anat. Rec. **83**:255, 1942. (*d*) Zettelman, H. J.: Am. J. Obst. & Gynec. **51**:241, 1946. (*e*) Gruenwald.[367b]

fetus and lead to aspiration of contents of the amniotic sac, future respiration may be impaired in one or both of two developments: One is mechanical, solid or fatty material filling or lining the air spaces [369]; the other is a fetal pneumonia which is caused in rare cases by the aspirated material. The manner in which the lung tissue reacts to these foreign substances varies greatly.[207b] In addition to the irritation caused by sterile material there may be a congenital bacterial pneumonia which also occurs only after aspiration of contents of the amniotic sac.[370] During periods of anoxia the fetus may discharge meconium, which will stain the vernix caseosa yellow.[371] Even the aspiration of meconium or of yellow vernix often fails to cause an inflammatory reaction of the alveoli.

SKELETON AND EXTREMITIES

Several reviews of the tremendous variety of skeletal malformations are available.[372] The developmental mechanism has been investigated in a relatively small number of these, and only this will be discussed here.

Duplication of entire extremities or large parts has repeatedly been produced in embryologic transplantation experiments in forms comparable with those occurring in man and other mammals, and the mechanisms of both kinds may well be similar in some respects. A single embryonic limb bud produces a double limb when transplanted in certain abnormal positions, and it has been concluded that the normal limb bud has an inherent tendency toward duplication which is normally suppressed.[373]

Excessive or reduced size of extremities may develop in several ways. It may be due to a somatic mutation, as has been assumed particularly when it is part of a general difference in size between the two sides of the body. A good example has recently been observed in a chicken.[65c] More references to similar lateral differences and other mosaics believed to be caused by somatic mutation may be found

369. Farber and Sweet.[207a] Nelson, W. E., and Smith, L. W.: J. Pediat. **26**:36, 1945.

370. Johnson, W. C., and Meyer, J. R.: Am. J. Obst. & Gynec. **9**:151, 1925. Hook, H., and Katz, K.: Virchows Arch. f. path. Anat. **267**:571, 1928. Kaldor, J.: Am. J. Obst. & Gynec. **25**:113, 1933. Bufe, W.: Ztschr. f. Geburtsh. u. Gynäk. **113**:265, 1936. Benner, M. C.: Arch. Path. **29**:455, 1940.

371. Clifford, S. H.: Am. J. Dis. Child. **69**:327, 1945.

372. (*a*) Aschner, B., and Engelmann, G.: Konstitutionspathologie in der Orthopädie, Berlin, Julius Springer, 1928. (*b*) Brandt, W.: Die Entstehungsursachen der Gliedmassenmissbildungen und ihre Bedeutung für das Vererbungsproblem beim Menschen, Leipzig, Johann Ambrosius Barth, 1937. Gruber.[74]

373. Brandt, W.: Arch. f. Entwcklngsmechn. d. Organ. **106**:193, 1925. Swett, F. H.: J. Exper. Zool. **44**:419, 1926.

in textbooks of genetics and in Hollander's review.[65b]   Another cause of abnormal size of extremities or of parts of them, with normal proportions within the part concerned, has been discussed by Politzer.[374] It is recalled that the embryonic limb buds develop as harmonious equipotential systems; that is, their cells have equal potencies, and the whole primordium develops into a harmonious limb, to a high degree independent of the amount of tissue present.   Increased or decreased size of the whole will thus produce a limb of abnormal size but normal proportions.   As a limb develops and its various parts are determined in its tissue, new harmonious equipotential systems develop for smaller parts, such as the forearm, the hand, the joints and the digits.   At any of these stages an abnormal amount of tissue will produce parts that are of normal proportions within themselves but out of proportion with the rest of the body.   In addition to obvious abnormalities of size, another malformation has been explained on this basis, which at the first glance does not seem to have anything in common with these, namely, congenital dislocation of the hip joint. Braus [375] made experiments in amphibians which indicate that at certain periods there exist independent harmonious equipotential systems for the two components of the joint.   He showed that in congenital dislocation the fossa is in itself well proportioned in its structure but is too small for the head of the femur and that a reduction of the amount of tissue available for the fossa brings about a condition comparable with the human malformation.   It is well known that in man dislocation of the hip joint is familial, and Faber [376] described a lesser degree occurring in affected families, which he called dysplasia. It produces no dislocation but can be detected by roentgenogram.

Many defects of the extremities, of varying extent and appearance, have long been regarded as the results of intrauterine amputation by amniotic bands and adhesions.   Many authors still adhere to this concept even though strong arguments have been brought forward in favor of a different origin of all or most of these defects.   Streeter [76] emphasizes the histologic abnormalities of the tissues at and near the site of the defect.   He assumes that primary and intrinsic abnormalities of the tissues, and not mechanical action of amniotic adhesions, are responsible, and that adhesions are sequelae of the fundamental abnormalities.   In the same year Ombrédanne and Lacassie [295] arrived at similar conclusions when studying clinical cases.   They point to various associated lesions, such as syndactyly, scars of the skin and the tongue, skeletal defects of the nonamputated parts, pseudarthroses and the

374. Politzer, G.: Beitr. z. path. Anat. u. z. allg. Path. **100**:273, 1938.

375. Braus, H.: Arch. f. Entwcklngsmechn. d. Organ. **30**:459, 1910; München. med. Wchnschr. **57**:1742, 1910.

376. Faber, A.: Ztschr. f. Orthop. **66**:140, 1937.

frequent symmetric locations of lesions. These cannot be satisfactorily referred to amniotic adhesions. On the other hand, the authors emphasize the occurrence of ulcers, which may be superficial or deep. One case is presented in which at birth an ulcer involved a large part of the forearm, healing with a considerable defect. This is considered as an unusually late occurrence of the disease which is called intra-uterine ulcerative disease *(maladie ulcéreuse intrautérine)*. Amniotic bands are assumed to be adhesions developing in the course of the disease. Other reports of intrauterine defects of the skin have been reviewed in an earlier section of this paper. Seitz [77] also assumes a primary abnormality affecting the fetal tissues to account for the development of amniotic bands. The abnormality may be either intrinsic or environmental. Gruber [74] has reviewed much of the older literature concerning amniotic amputations. He accepts amniotic bands as the cause of the defect in some cases. In support of the amputation theory, Hellner [79] put ligatures around fetal extremities in animal experiments. The defects resembled those observed in nature, and the amputated parts rapidly disappeared by autolysis. Movers [377] states that not only defects of the extremities but also severe distortions of large parts of the body are not satisfactorily explained by amniotic adhesions. Taylor Gorostiaga and Lede [78] recently supported Streeter's view with histologic observations made in a pertinent case. In connection with fetal ulcers and amputations, intrauterine fractures of long bones occur.[378] One may wonder whether these are not pathologic fractures of diseased bones.

In favor of an intrinsic cause of the defects in question is the occasional familial occurrence. A good example is a family in which several children had so-called amputations of forearms and legs of identical form, bilateral and symmetric. Koehler [75] has reviewed several conflicting accounts of this family. According to the version which he considers most reliable, the mother of 6 defective and 6 normal children was the father's niece or sister. In spite of this obvious familial background, one of the authors reporting on this family considers the malformations as amniotic band amputations.[379]

The morphologic aspects and the development of a condition possibly related to those just reviewed were described by Greene and Saxton.[380] Brachydactyly and other defects occur in rabbits as simple recessive mutations. In the embryos, dilatation of blood vessels, hemorrhage and eventually necrosis and sloughing are observed. Somewhat similar is a group of malformations which have been investigated in great

377 Movers, F.: Arch. f. Gynäk. **168**:22, 1939.

378. Granzow, J.: Zentralbl. f. Gynäk. **55**:3458, 1931.

379. Joesting, H.: Therap. Beitr. **10**:51, 1933.

380. Greene, H. S. N., and Saxton, J. A., Jr.: J. Exper. Med. **69**:301, 1939.

detail, namely, hereditary defects of limbs and other parts of the body in a strain of mice. Early investigations revealed blisters and hemorrhages in the regions where malformations occur.[381] The complete sequence of events has been described by Plagens [382] and Bonnevie.[334] The authors agree in the essential points, and in the following presentation the description of Bonnevie will be followed. As has been mentioned in the description of ocular malformations occurring in the same strain, an excess of cerebrospinal fluid escapes from the brain and moves under the epidermis in the form of blisters ("myelencephalic blebs") until it reaches points where it can apparently go no farther. This happens frequently in the limb buds, and malformations ranging from clubfeet and syndactyly to defects resembling the so-called amniotic amputations result from the mechanical interference of these blisters which later become hemorrhagic.

There are various forms of micromelia, or disproportionate dwarfism (as against proportionate dwarfism), in which the parts of the skeleton of the extremities are present but deformed. These abnormalities have been studied most extensively in fowl.[170] Various types of micromelia result in fowl from a deficiency of manganese [363] or riboflavin [174] or from selenium poisoning [125] during embryonic life. Typical chondrodystrophy occurs sporadically [384] or as a hereditary trait.[385] Several mutations produce similar but quantitatively different forms of chondrodystrophy in heterozygous embryos and more severe changes, namely, early death or phocomelia, in homozygous embryos. Of these mutations, the Creeper fowl has been studied by several groups of workers. The results are of particular interest, because it has been shown that the chondrodystrophy or the phocomelia of these specimens closely resembles the human malformation.[385c] In man and fowl, chondrodystrophy is characterized by short, thick, curved bones of the extremities, while the vertebral column usually shows minor changes, if any, and the skull is normal or has a shortened base. The details of the malformation of the long bones vary considerably, and

381. Bagg, H. J., and Little, C. C.: Am. J. Anat. **33**:119, 1924. Bagg, H. J.: ibid. **43**:167, 1929.

382. Plagens, J. M.: J. Morphol. **55**:151, 1933.

383. Byerly, Titus, Ellis and Landauer.[166] Landauer.[167] Lyons and Insko.[168] Caskey and Norris.[169]

384. (*a*) Landauer, W., and Dunn, L. C.: Proc. Soc. Exper. Biol. & Med. **23**:562, 1926. (*b*) Landauer, W.: Arch. f. Entwcklngsmechn. d. Organ. **110**:195, 1927.

385. (*a*) Landauer, W., and Dunn, L. C.: J. Genetics **23**:397, 1930. (*b*) Landauer, W.: Ztschr. f. mikr.-anat. Forsch. **25**:115, 1931; (*c*) **32**:359, 1933. (*d*) J. Genetics **31**:237, 1935; (*e*) Bulletin 233, Storrs Agricultural Experiment Station, 1939, p. 1. (*f*) Am. Naturalist **76**:308, 1942. (*g*) Lamoureux, F. W.: J. Hered. **33**:275, 1942.

various subtypes have been described. A good summary of findings in human chondrodystrophy and the theories concerning it has been given by Gruber.[74] The morphologic aspects and the development of hereditary chondrodystrophy of cattle have been described by Crew [386] and Mohr.[1d] Only the embryogenesis of the hereditary chondrodystrophy of fowl has been described.[387] In early stages the cartilaginous parts of the skeleton are of normal size and proportions. Later on, growth is retarded. The various processes involved in the growth of cartilage and its replacement by bone are not properly coordinated. Enchondral ossification starts at the usual time but soon lags severely behind the normal process in extent. Perichondral ossification at times exceeds the normal rate, and this probably causes the large size of the fibula in man and fowl, by preventing partial involution of the structure. Connective tissue is frequently observed to extend from the periosteum into the epiphysis *(Perioststreifen)* in man and, in sporadic cases, in fowl, and occasionally in Creeper chicks. Landauer [384b] found that this formation does not cause curvatures of long bones by unilateral inhibition of epiphysial growth as had been supposed, but is preceded by them.

Many old theories holding that chondrodystrophy is caused by extrinsic agents during embryonic life (Gruber [74]) have been discarded. In part of the cases it is caused by genetic factors; the cause of sporadic chondrodystrophy is unknown. Landauer,[388] in accordance with previous investigators, concluded that there are no abnormalities of endocrine organs of the embryo which could account for the malformation. Phocomelia of homozygous Creeper embryos and chondrodystrophy of heterozygous birds are degrees of the same basic type of disturbance.[385c] However, it is acknowledged that this does not necessarily hold for all cases of phocomelia.

Embryologic experiments with Creeper fowl have shown that in limb buds of homozygous or heterozygous Creeper embryos transplanted to genetically normal hosts previous to the development of their skeletons, abnormalities develop according to their own genotype and independent of the host.[389] This shows that the malformation is not caused by other parts of the embryo acting on the primordia of the limbs. Several lines of evidence, in addition to the general principles discussed in part I, suggest that the Creeper factor acts by reducing developmental activity during a critical stage. The action of this genetic factor and that of selenium poisoning are cumulative, and the latter is probably

---

386. Crew, F. A. E.: Proc. Roy. Soc., London, s.B **95**:228, 1923.

387. Landauer (footnotes 384 *b* and 385 *b* and *c*).

388. Landauer, W.: Virchows Arch. f. path. Anat. **271**:534, 1929.

389 (*a*) Hamburger, V.: Physiol. Zoöl. **14**:355, 1941. (*b*) Rudnick, D.: J. Exper. Zool. **100**:1, 1945.

represented by a retardation of metabolism.[49]   When genetically normal limb buds develop in vitro in a growth-restricting medium they yield deformities resembling those of Creeper embryos.[390]   A disturbance of the metabolism of older chondrodystrophic chick embryos has been reported by Patton,[391] who found that the tissues of these embryos contain in later stages significantly less aminoacetic acid than do those of normal embryos.

In embryonic manganese deficiency of fowl the long bones show, in addition to a reduction of length, histologic abnormalities of the cartilage and a matrix containing fragments of degenerated cartilage cells in place of periosteal bone.   If the involved chickens receive an adequate diet, the abnormal tissue is replaced by normal bone.[167]

A mutation of fowl causing a short upper beak and short long bones is on record.[392]   In all these forms, including chondrodystrophy, the legs are more severely deformed than the wings, and the distal bones more than the proximal ones.[392]

A nutritional deficiency, perhaps of riboflavin, during gestation produces various skeletal and other malformations in rat embryos.[393] The abnormal condition is determined in the cartilaginous stage of skeletal development, and addition of the missing substance to the mother's diet prevents malformations only if it is made before the fourteenth day of gestation.[185]

Micromelia not conforming to any established type and affecting the limbs of one individual to different degrees may result from roentgen irradiation of embryos,[394] though perhaps less frequently than malformations of the brain or the eyes.

Brandt [395] found it difficult to produce phocomelia experimentally in amphibians, because, after the excision of tissue, either the limb is absent or it regenerates and develops normally.   Only by long-lasting interference such as occurs through implantation of other tissue into the limb bud could the desired effect be obtained.   Gabriel [118] produced dwarf limbs in chick embryos by local application of colchicine.

In some of the so-called amniotic amputations the condition is really one of irregular micromelia rather than a terminal defect, as traces of digits are present.[396]

390. Fell, H. B., and Landauer, W.: Proc. Roy. Soc., London, s.B **118**:133, 1935.

391. Patton, A. R.: J. Nutrition **13**:123, 1937.

392. Landauer, W.: Genetics **26**:426, 1941.

393. Footnote 185. Warkany and Schraffenberger.[185]

394. Murphy.[21] Feldweg.[95c] Flaskamp.[95f]

395. Brandt, W.: J. Exper. Biol. **20**:117, 1944.

396. Ombrédanne, L., in Ombrédanne, L., and Mathieu, P.: Traité de chirurgie orthopédique, Paris, Masson & Cie, 1937, vol. 1, p. 23.

Hereditary deformities of the legs of mice, produced by defects of the tibias, have been thoroughly examined by Hovelacque and Noel.[397] In the embryo the condensations of mesenchyme which initiate the formation of skeletal parts are present for the tibias as usual. However, instead of differentiating into cartilage, the greater part of the tibial condensation forms a fibrous ligament. Later on, the fibula is severely curved. However, it was observed that even in externally normal mice of the same strain, which had been considered as normal overlaps, the tibias might be defective, though the fibulas were without deformity. Defects of the tibias have also been found in polydactylous guinea pigs carrying the lethal gene Px.[236]

Hereditary polydactyly was the subject of one of the earliest systematic investigations of the embryonic development of a malformation. In 1908 Kaufmann-Wolf[398] and Braus[399] examined polydactylous chick embryos and observed that an enlargement of the foot represented a partial reduplication. The toes developing in such excessive tissue form part of a mirror image of the normal foot at its tibial border. Gabriel,[118] also, believes that polydactyly is an expression of reduplication of the limb. By treatment with colchicine he reduced the number of digits in normal and polydactylous chick embryos. Cole[400] investigated a lethal trait producing severe polydactyly of all extremities, ectopia viscerum and malformations of the face in fowl. At one hundred and ten hours of incubation the limb buds are deformed, and homozygous embryos die at six to eight days of incubation.

Wright[401] examined in guinea pigs the inheritance of a type of polydactyly which produces in heterozygous embryos five digits instead of the usual four on the forefeet and three on the hindfeet. Homozygous embryos have excessive polydactyly, with ten and more digits on a foot, and multiple other malformations, which cause death usually on the twenty-seventh day in utero or, rarely, immediately after birth. Scott[236b] found in newborn homozygotes, aside from approximately twice the normal number of digits, clubfoot, absence of the tibia, microphthalmia, malformation of the brain and, in part of the cases, harelip or absence of nipples. From the study of the embryogeny of these malformations[236a] it was concluded that the gene Px causes in homozygous form excessive growth and retarded morphogenesis about the age of 17½ days in utero, affecting rapidly growing parts more than others. The resulting abnormal correlation of developmental

397. Hovelacque, A.: Bull. biol., 1920, supp. 3, p. 1. Hovelacque, A., and Noel, R.: ibid. **57**:133, 1923.
398. Kaufmann-Wolf, M.: Morphol. Jahrb. **38**:471, 1908.
399. Braus, H.: München. med. Wchnschr. **55**:386, 1908.
400. Cole, R. K.: Poultry Sc. **18**:403, 1939.
401. Wright, S.: Genetics **20**:84, 1935.

processes, further augmented by a normal onset of histogenesis in the morphogenetically retarded parts, accounts for most of the disturbances. The development of polydactyly in this mutation may well be related in its mechanism to that found in various other mammals, including man.[236b]

It remains to mention several abnormalities concerning mainly the histogenesis of the skeleton. It was just mentioned that faulty differentiation in an early phase, namely, formation of connective tissue instead of cartilage, accounts for hereditary absence of the tibia in mice. Grüneberg[1r] points out that a disturbance in a comparable stage, though in the opposite sense, produces flexed tails and similar malformations of vertebrae in the trunk region in a mutation of mice: The intervertebral disks are partly transformed into cartilage instead of fibrous connective tissue.

The ossification of the cartilaginous parts of the skeleton is subject to a variety of abnormalities. These are too complex to be described here in detail. One example is chondrodystrophy, which has already been discussed. A hereditary failure of bone resorption in mice (the gray-lethal mutation) leads to deformities of bones and prevents eruption of teeth.[402] Transplantation experiments showed that in normal hosts gray-lethal bones develop nearly normally; in gray-lethal hosts they retain their abnormal features. Bones of normal mice transplanted to gray-lethal hosts sometimes show deformities resembling the gray-lethal condition; transplanted to normal hosts, they remain normal. This suggests an abnormal action of the tissue fluids of the gray-lethal host.[403] Administration of parathyroid extract to gray-lethal mice causes much resorption of bone. These mice tolerate larger quantities of parathyroid extract than would normal mice. However, in spite of the improvement of the condition of their bones, the mice are not cured by the treatment. Since they show no other evidence of deficiency of the parathyroid glands, it has been concluded that either their osteoclasts require an abnormally high concentration of the hormone or the hormone is excreted too rapidly, or both.[404]

With regard to chickens, a lethal factor *"sticky"* has been briefly described,[405] which was so named because the amniotic and allantoid fluids are viscid. The embryos have edema and soft bones, and do not hatch. The bone changes are described as similar to those associated with experimental vitamin D deficiency,[406] but more study is necessary to elucidate the relationship of the two disturbances.

402. Grüneberg, H.: Proc. Roy. Soc., London, s.B **118**:321, 1935.
403. Barnicot, N. A.: Am. J. Anat. **68**:497, 1941.
404. Barnicot, N. A.: J. Anat. **79**:83, 1945.
405 Byerly, T. C., and Jull, M. A.: J. Exper. Zool. **62**:489, 1932.
406. Byerly, T. C.: Poultry Sc. **10**:404, 1931.

A great deal of confusion exists in the literature concerning experimental and human vitamin deficiencies of the embryo and their effects on the skeleton. Only a few reports will be mentioned here, and in all cases the nomenclature of the respective author will be used. Ingier [188a] published a study of Barlow's disease (scurvy) produced in guinea pig embryos by feeding the mother a diet of oats and water. In the report, scurvy, rickets, osteomalacia and osteogenesis imperfecta are mentioned, and no clearcut identification of the changes is made. Reyher, Walkhoff and Walkhoff [188b] describe vitamin C deficiency of guinea pigs, resulting in scurvy of the mothers and the embryos. The changes are compared with those noted in a newborn baby of their own observation. Maxwell, Hu and Turnbull [193b] observed a mother with osteomalacia and her baby with fetal rickets, and in their reports, stress the close relationship of the two diseases. Warkany [187] studied the effect of a maternal rachitogenic diet on the skeletons of rat embryos and observed changes similar to, but not identical with, rickets. He calls attention to the fact that the "diet" of the fetus is not the same as that of the mother.

Strontium treatment of pregnant rabbits produces in the fetal skeleton "pseudorickets" with increased apposition and decreased resorption of bone.[130]

The skeletal manifestations of fetal syphilis are too well known to be reviewed; they are described in textbooks of pathology. Postnatal developmental disturbances of the skeleton will be briefly referred to in part III of this review.

UROGENITAL TRACT

The explanation of malformations of the urogenital tract is facilitated by knowledge of several phases of its developmental physiology. This holds particularly for the kidney. Long before experimental evidence was at hand, Fischel [407] suggested that nephrons differentiate in the metanephric blastema only after their differentiation has been properly induced by the ureteric bud which, by its own differentiation, forms the collecting tubules, the renal pelvis and the ureter. This was assumed because neither of these two components of the kidney was ever found alone, or separated from the other (except perhaps small rudiments of ureters without a pelvis). Experimental elimination of the ureteric bud of the chick embryo has since furnished ample confirmation of Fischel's hypothesis.[408] These investigations, as well as

407. Fischel, A.: Die Bedeutung der entwicklungsmechanischen Forschung für die Embryologie und Pathologie des Menschen, in Vorträge und Aufsätze über Entwicklungsmechanik der Organismen, Leipzig, W. Engelmann, 1912, no. 16.

408. Boyden, E. A.: J. Exper. Zool. 40:437, 1924. Proc. Soc. Exper. Biol. & Med. 24:572, 1927. Gruenwald, P.: Arch. f. Entwcklngsmechn. d. Organ. 136:786, 1937.

examinations of serial sections of suitable embryonic human specimens,[409] have made it clear that in man as well as in the chick aplasia
of the ureteric bud results in absence of the entire organ, including
also the derivatives of the metanephric blastema. The latter is present
as usual but fails to differentiate in the absence of the inductor.

In mice, the gene which produces malformations of extremities,
jaws and eyes, due to migrating blebs of fluid (see other sections of
this review), is also responsible for renal malformations.[410] In the
embryo the ureteric bud may be reduced or absent. If it is present, it
does not branch and thus fails to induce differentiation of kidney tissue
in the metanephric blastema.[411] In another mutation of the mouse [276e]
it was found that complete absence of the ureteric bud or its failure to
branch properly is followed by a complete lack of differentiation of
nephrons even though the blastema is present.[283]

Various theories of the development of polycystic kidneys have been
reviewed by Gruber.[412] According to one theory, the condition is
caused by an abnormality of the union of the two components of the
kidney, resulting in lack of drainage and subsequent distention of some
nephrons. Actually it has been demonstrated that the interruption of
the lumen does not usually occur at the point at which the nephron
fuses with the collecting tubule. Roos's [413] own specimen, which its
investigator thought to be proof of the nonunion theory, shows atresia
at the junction of Bowman's capsule and proximal convoluted tubule,
far from the point of union in the embryo. Moolten [350d] speaks of the
nephron as migrating to join with the collecting tubule and suggests
that this process may have failed in cystic kidneys; no such migration
actually occurs. Berner's [414] detailed study of a large number of cystic
kidneys demonstrated long ago that the interruption of the tubule may
occur at any point. This indicates either that there are several mechanisms of origin affecting different parts of the tubules or that one
mechanism may affect various points, the particular one depending
perhaps on the timing of its action.

Occlusion of the lumen is not generally accepted as the principal
lesion of polycystic kidneys. Some authors assume that active overgrowth rather than distention accounts for cyst formation.[412] Moolten [350d] advocates the interpretation of cystic liver and kidneys as a

409. Boyden, E. A.: Anat. Rec. **52:**325, 1932. Gruenwald, P.: ibid. **75:**237,
1939.

410. Bagg, H. J.: Am. J. Anat. **36:**275, 1926.

411. Brown, A. L.: Am. J. Anat. **47:**117, 1931.

412. Gruber, G. B., in Schwalbe,[256] 1927, pt. 3, sect. 3, p. 157.

413. Roos, A.: Am. J. Dis. Child. **61:**116, 1941.

414. Berner, O.: Die Cystenniere: Studien über ihre pathologische Anatomie,
Jena, Gustav Fischer, 1913.

type of hamartiosis, or tissue malformation "based on a disturbance in organizer action" which manifests itself as cystlike gigantism of bile ducts or renal tubules. Actually there is no proof of this. Occlusion, as well as overgrowth of tubules, may be determined by interaction of adjacent tissues, but this is no more likely than an inherent deficiency of the tissue, perhaps determined by direct gene action. An old theory having in view obstruction of renal tubules on an inflammatory basis [412] has not been substantiated by histologic examination in the great majority of cases and has generally been abandoned.

Kampmeier [415] found that some of the earliest formed nephrons, located in the innermost portion of the embryonic renal cortex, degenerate before birth, and believes that their remnants may form cysts. This may explain cysts of the inner portions of the cortex, but it cannot account for the polycystic kidney in which the peripheral nephrons are also, or sometimes predominantly, affected, even with the assumption that the cysts of the inner portion of the cortex compress other tubules and that this leads to the formation of additional cysts.[416]

Dystopic kidneys are most commonly pelvic or fused kidneys. To understand their development it is necessary to know that the kidneys are at first located in the sacral region and that they move upward by a complicated process.[417] If this movement fails to occur, a pelvic kidney results. On the other hand, a slight medial deviation of the renal primordia is sufficient to bring them in contact with each other, producing a horseshoe kidney, particularly since the kidneys are normally close to each other at one point of their upward route.[418] Other displacements of the kidneys are less frequent, and their explanation is unknown. Among these is the rare one, in which the right kidney is displaced upward into the thorax, which occurs in combination with diaphragmatic hernia.[419] The arteries supplying dystopic kidneys arise from the aorta or its major branches at abnormal points corresponding to the locations of the kidneys. It was believed that the abnormal blood supply might be the cause of the dystopia of the kidneys, but investigators now know that this was erroneous, since the permanent renal vessels develop only after the kidneys have reached their final locations. The abnormal origin of the vessels is a consequence rather than the cause of dystopic kidneys.

Just as the permanent kidney depends in its development on the ureteric bud as an inductor of the metanephric blastema, the mesoneph-

415. Kampmeier, O. F.: Surg., Gynec. & Obst. **36**:208, 1923.

416. McKenna, C. M., and Kampmeier, O. F.: Tr. Am. A. Genito-Urin. Surgeons **26**:337, 1933.

417. Gruenwald, P.: Anat. Rec. **85**:163, 1943.

418. Lewis, F. T., and Papez, J. W.: Anat. Rec. **9**:105, 1915. Gruenwald.[417]

419. von Mikulicz-Radetzki, F.: Zentralbl. f. Gynäk. **46**:1718, 1922.

ros depends on the wolffian duct as an inductor of its differentiation.[408] In addition, the wolffian duct acts as a guide for the growing müllerian duct.[420] However, the gonads and the adrenal cortex do not depend on any of the nearby organs for proper differentiation.[408] The development of the urogenital tract of the chick embryo after experimental elimination of a wolffian duct shows a combination of defects, owing to the leading role of that duct. The combination is an exact duplication of the most common combination of malformations observed in man and other mammals of either sex.[421] In experimental embryos mesonephrons and müllerian ducts are absent when there is no wolffian duct. In addition, the ureteric bud is missing, since it is normally formed from the caudal part of the wolffian duct, and with it the entire kidney. In the male the result is absence of one kidney and part or all of the epididymis, as well as of the ductus deferens and the seminal vesicle on the same side. In the female the same disturbance results in absence of a kidney and the corresponding half of the uterus (in man: uterus unicornis of the other side) with part or all of the tube. Gonads and adrenal glands are not affected. This is an instructive example of the action of various types of correlation which normally insure proper development; a minute lesion at one point (the growing end of the wolffian duct) is followed by a syndrome of defects.

INTERSEXUALITY AND HERMAPHRODITISM

The investigation of sexual intergrades has in the past yielded innumerable suggestions for their explanation and nomenclature. However, it was not until modern genetic and endocrinologic animal experiments had produced much information that a satisfactory concept could be evolved. One of the most extensively used systems of classification distinguished among individuals with partly male and partly female structural characters true hermaphrodites and pseudohermaphrodites. The former have both testicular and ovarian tissue, either as ovotestes or as one testis and one ovary. Pseudohermaphrodites have uniform gonads, but part of the other sex organs are differentiated according to the opposite sex. All pseudohermaphrodites and the hermaphrodites with ovotestes are now often referred to as intersexes. Lateral hermaphrodites, and particularly those in which all genital organs show a striking lateral difference, are most probably due to a somatic mutation which results in a different genetic constitution of the two sides.

The existence of two kinds of intersexuality, genetic and hormonal, and the chromosomal mechanism of the former were discussed in part I. It remains to review briefly the theories of their development.

420. Gruenwald, P.: Anat. Rec. **81**:1, 1941.
421. Gruenwald, P.: Beitr. z. path. Anat. u. z. allg. Path. **100**:309, 1938.

All persons have in their genes male and female determining factors, with one kind outweighing the other. It is generally believed that genetic intersexes are those in which there is a low degree of preponderance of one sex, insufficient to assure normal development. There are two theories concerning the manner in which structural intersexuality develops in this instance. Goldschmidt [61] concludes from his genetic work with arthropods, and assumes for vertebrates, that 'intersexes begin their development in a normal fashion in the direction of the predominant sex but that they reach a turning point if the predominance of one sex is below a certain value. If the predominance is but slightly below the limit of normal, the turning point is at a late stage; the smaller (and the farther from normal) the predominance, the earlier is the turning point. After that point development proceeds entirely in the direction of the opposite sex. The result depends on the amount of irrevocable differentiation which has occurred prior to the turning. What has definitely developed in the direction of one sex at this time mixes with the remaining traits which subsequently differentiate according to the other sex. Goldschmidt presents several not quite convincing arguments to show that male intersexes (i.e., those beginning with a male phase before the turning point) do not exist in man and other mammals. One of the principal arguments is the inability of the testis to form an ovarian cortex in the event of transformation; this has since been shown to be incorrect.[422] Severinghaus found in a human intersex chromosomes characteristic of a male (X and Y), and Witschi [160] concludes from statistical data that the intersexes in his material develop at the expense of the males. Moszkowicz,[423] who is otherwise an ardent follower of Goldschmidt, assumes on morphologic grounds that male intersexes exist.

Apart from the question of male intersexes, the theory of Goldschmidt has been severely criticized by Bridges,[60] who developed the theory of genic balance. This is also based on the assumption of an abnormal quantitative relation of male and female sex factors, leaving the margin of either male or female factors insufficient to determine normal development. However, it is assumed that the individual is intersexual and develops as such at all times, since the genic constitution does not change. It is pointed out that Goldschmidt's results in arthropods can also be interpreted according to this concept.

Goldschmidt's theory was a potent stimulus for the examination and interpretation of the structure of human and mammalian intersexes, as is evident from the review of Moszkowicz.[423] However, it has not been a successful working hypothesis. If it were correct, all

---

422. Gruenwald, P.: Am. J. Anat. **70**:359, 1942.

423 Moszkowicz, L.: Ergebn. d. allg. Path. u. path. Anat. **31**:236, 1936.

forms should fall into one linear succession of degrees according to the times of turning from female to male development. According to Moszkowicz, two such series of forms should exist, including also those resulting from transformation of primarily male individuals. However, the observed forms cannot be readily classified in this manner. Moreover, in many attempts to determine the turning point by examination of the final condition, the same mistake has been made which occurred in many attempts of older teratologists to determine in a similar manner the latest time at which a given malformation could have started *(teratogenetische Terminationsperiode)*. The morphologic differentiation of an organ at various stages of its normal development was taken as a criterion of its ability to deviate from the normal course and produce the malformation in question. Experimental embryology has definitely established the fact that in some cases a given step of *differentiation may be determined before it is visible and that in others* a fully developed differentiation may 'change under certain conditions. As illustrations from the field of sex differentiation which show that it is impossible to determine a turning point with the aid of descriptive embryology, the following examples may be given: In rats ovarian follicles have been transformed into structures resembling seminiferous tubules long after birth by administration of testosterone propionate.[424] Genetically male chick embryos which had been completely feminized by estrogenic treatment in early embryonic stages changed completely back to their genetic sex after hatching, their gonads, which were ovaries at hatching, becoming testes with spermatogenesis.[155] These considerations invalidate all attempts to support Goldschmidt's theory by reconstructing the turning point in human cases of intersexuality.

Hormonal intersexuality occurs occasionally through the action of hormone-secreting tumors, and it has been produced repeatedly in the laboratory by the administration of androgenic or estrogenic substances to embryos. In both types the result depends, as is to be expected, on the kind, the concentration and the time of action of the substance administered. Another factor of great importance is the genetic background. Not only do corresponding embryonic primordia of genetic males and females react differently to hormonal stimulation, but various traits are fixed and resistant to changes to different degrees in different species.

A condition related to hormonal intersexuality, in which most of these factors are of importance, is that of the freemartin; in this instance one of heterozygous twins is obviously influenced by substances transmitted through anastomoses of the circulatory system from the other twin. This has been discussed in part I, where it was mentioned that

424. Marx, L.: J. Exper. Zool. **91**:365, 1942.

the nature of the active substance and its relationship to sex hormones is not known.

When an androgenic or an estrogenic substance is introduced into an individual, it acts by inhibiting some traits which would normally develop and by stimulating others which would otherwise regress or fail to develop. Burns[425] has emphasized that sex characters fall into two groups, one in which there are two different structures, one of which develops in the male and the other in the female ("alternative characters"), and another in which the same primordium follows a course of progressive development in the male which is different from that followed in the female ("nonalternative characters"). It is obvious that if a primordium of the former group has disappeared in accordance with sexual differentiation (e.g., the wolffian duct in the female), it cannot be brought back into existence by hormonal reversal of sex. In regard to other primordia of organs which develop in both sexes but in different ways, it is extremely difficult to predict the extent to which transformation is possible at a given stage. Morphology alone cannot answer this question, as was discussed in a foregoing paragraph.

Witschi[160] assumes, on the basis of experiments with amphibians, that the transformation of sex organs by action of hormones occurs by inhibition of structures of the sex opposite to that to which the newly introduced hormone pertains and subsequent compensatory growth of those of the other sex. The latter is not caused by direct stimulation by the substance administered. In agreement with this is the experience that if the left ovary of the chicken is removed, the right gonad (which never has an ovarian cortex) hypertrophies and develops into a testis, with concomitant masculinization.[426] Witschi extends his view to all vertebrates. It remains to be seen whether all findings in mammals are in accord with this.

Embryologic experiments have not confirmed the expected division of sex hormones into strictly male and female ones. Stimulation of structures of the opposite sex has repeatedly been found. However, Burns[149b] holds that this occurs only when excessive doses are used. The extent of stimulation of the same organ (e. g., the prostate) differs in genetic males and females, apparently under the influence of the genetic constitution.[149] The morphologic results of the administration of androgenic and estrogenic substances to embryos have repeatedly been reviewed.[427] They are too complex to be described here.

425. Burns, R. K., Jr.: Am. Naturalist **72**:207, 1938.

426. Domm, L. V.: Proc. Soc. Exper. Biol. & Med. **26**:338, 1929.

427. (*a*) Domm, L. V., in Allen, Danforth and Doisy,[64] p. 227. (*b*) Burns.[149b] (*c*) Burns, R. K., Jr., in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1942, vol. 10, p. 27. (*d*) Greene and others.[151b] Moore.[153]

Information on the interaction of genetic and hormonal factors has changed modern views on sex development. It was assumed until a few years ago that the genetic mechanism determines the sex of the gonad and with it the type of hormone to be· secreted. The other, so-called secondary sex characters were believed to be determined by those hormones, and not by the genes directly. This concept evolved from the examination of relatively late, namely, postnatal, changes in which the influence of the genetic structure is overshadowed by conspicuous hormone actions. Experiments with embryos, as well as continued work with older stages, have demonstrated the fundamental importance of the genotype in the development, the maintenance and the reaction of sexually differentiated traits, including also the so-called secondary characters. This has been summarized by Moore,[153] who comes to the conclusion that in the embryo there is a long period of active sexual differentiation during which the gonads do not produce hormones and are not able to do so even when stimulated by the administration of gonadotropic substances. During this period the principal features of the sex organs differentiate apparently by direct gene action. However, during this same period the organs are susceptible to the action of artificially introduced estrogen or androgen. Even later on the action of hormones of the opposite sex is counteracted to some extent by the genotype. This is illustrated by the development of genetically male chick embryos which are feminized by estrogen injected into the egg to the extent that they have normal ovaries and female genitalia. In spite of this morphologically complete transformation, they revert to their genetic sex after hatching if treatment is discontinued, and are found later on to have functional testes.[155]

Danforth [428] has shown in an instructive table the varying extent to which genes and hormonal influences affect the plumage of several species and breeds of birds.

With these factors of genetic and hormonal determination of sex development in mind, one may attempt to interpret intersexuality as it is found in man and to formulate a basis for its management. It is clear from what has been said that a genetic intersex has no normal male or female sex. What some authors call the genetic sex of the individual, namely, the sex indicated by the number of sex chromosomes (either XX or XY), is of little value in these considerations, as it does not indicate the actual relation of male and female factors in the set of chromosomes. Imbalance of sex factors may go so far as to produce an individual of normal sex development and instincts opposite to the

428. Danforth, C. H., in Harvey Lectures, Baltimore, Williams & Wilkins Company, 1939, vol. 34, p. 246.

sex indicated by the mere number of sex chromosomes. This is an extreme which may not occur in man, but all intergrades between it and the normal may be expected.

The apparent combination of male and female bodily traits in the intersex had led many to assume an antagonism between male and female tendencies. In reality the only antagonism is that of the male and female genic sex factors, and this exists in normal individuals as well. In physical development a genetic intersex is as true to its own genotype as is any normal individual. It is futile and misleading to seek the "real" male or female sex in intersexes. Since one knows that the genic control which guides embryonic sex development also exists later on and that hormone action is but superimposed on it, it is unreasonable to attempt to produce a normal sex by giving hormones or by removing them (gonadectomy). Each intersex produces its appropriate hormones, no matter whether its gonads are testes or ovaries. To remove testes because they seem to be in opposition to a predominantly female external development means to castrate the person, and shows a fundamental lack of understanding.[164] It is also clear that attempts to determine the sex (in terms of male or female) of an intersexual child is futile, since no correlation has as yet been found which would permit one to determine the future psychic sex from somatic traits. For the same reason biopsy of the gonads is of no avail. It is therefore dangerous to do a plastic surgical operation on the genitalia of the infant with genetic inter-sexuality, since one cannot know at that time what the psychic sex will be.[429] It is necessary to declare an intersex as a male or a female, but this is arbitrary and has to be changed at puberty in many cases. The relationship between intersexuality and homosexuality is not clear.

Hormonal intersexuality offers to the physician an entirely different problem. When it develops in the embryo, it is usually due to hyperplasia or adenoma of the adrenal cortex,[163] which apparently produces an unidentified hormone. In the majority of cases females are affected, as is also true of postnatal "interrenalism." Elimination of the source of abnormal hormonal activity will allow the normal genetic sex to assert itself, and the structural changes will follow as far as is physically possible.

In the medical literature the principles outlined in the foregoing paragraphs have been increasingly appreciated during recent years. Novak [430] emphasizes the important role of genes in the early phases of development of the genital organs. Moszkowicz,[423] Schiller [164] and Greenhill and Schmitz [429] discuss the nature of the sex determination of genetic intersexes and warn against corrective procedures until the

429. Greenhill, J. P., and Schmitz, H. E.: West. J. Surg. **48**:36, 1940.
430. Novak, E.: J. A. M. A. **105**:413, 1935.

psychic sex manifests itself. Schiller stresses the grave consequences of gonadectomy, even in cases in which the sex of the gonads is seemingly opposite to that of the individual intersex. McCahey's [431] position is not so well founded on biologic principles. He divides intersexes into genetic males and females. This leads him to the statement that "erections of the phallus in genetic females are pathological" and that these must be prevented by excision of all testicular structures. It should be clear that erections of the phallus are normal in any individual, and if an intersex with female feelings resents them, it is because of the excessive size of the organ. That might be dealt with surgically after puberty, when there can be no doubt of the psychic sex, but under no circumstances by gonadectomy. McCahey uses as a criterion of genetic femaleness the presence of derivatives of the müllerian ducts, a criterion which is entirely unfounded.

The only genetic sex abnormality in which there can conceivably be antagonism between male and female parts is true lateral hermaphroditism. According to the only satisfactory explanation, this is a genetic mosaic, apparently due to a somatic mutation (see part I) which leaves the two halves of the body with different genotypes. The assumption that the entire body and not just the genital tract is affected is borne out by a corresponding lateral difference of the plumage of birds with a similar constitution.[427a] The fact that the halves of the body develop independent of each other points to the leading role of the genotype of the tissues, since the hormonal environment is the same. Gynandromorphism has been reviewed by Moszkowicz,[428] Witschi[160] and others. A recent report of a human case is that of Lattimer, Engle and Yeaw.[432]

CARDIOVASCULAR SYSTEM AND BLOOD

In spite of the large number and the intriguing features of cardiac malformations, no direct information concerning their developmental mechanisms is available. Several theories of the abnormalities of septation and of the relations of the large vessels to each other and the heart are on record.[433] The subject is too complex to be reviewed in brief, and the original articles should be consulted by those interested in this field. As a cause of cardiac malformations, maternal rubella occurring in the early months of pregnancy has been recognized,[217] but here, too, the mechanism is unknown. Fetal endocarditis as a possible cause has been suggested by some and rejected by others.[434] Farber and Hub-

431. McCahey, J. F.: Surg., Gynec. & Obst. **67**:646, 1938.

432. Lattimer, J. K.; Engle, E. T., and Yeaw, R. C.: J. Urol. **50**:481, 1945.

433. Spitzer, A.: Virchows Arch. f. path. Anat. **243**:81, 1923; **271**:226, 1929. Pernkopf, E., and Wirtinger, W.: Ztschr. f. Anat. u. Entwcklngsgesch. **100**:563, 1933. Lev, M., and Saphir, O.: J. Tech. Methods **17**:126, 1937.

434. Gross.[212] Weintraub and Himmelfarb.[213] Footnote 214.

bard [216b] point out that malformations of the heart may be divided into two groups, of which one, comprising severe disturbances of the structure, is caused by primarily abnormal development, whereas the other, consisting of stenoses or atresias of ostiums in otherwise normal hearts, may be due to endomyocarditis of the embryo. In some cases of the latter group, microscopic study reveals indications of a previous inflammation, such as myocardial fibrosis or areas of calcification.. Cardiac malformations occur in more than 20 per cent of all cases of mongolism.[141b] Their relationship to the suggested causes of this deficiency is unknown.

Remarkable success has recently been reported with the surgical treatment of certain malformations of the heart and large vessels.[435] This emphasizes the need for understanding and exact diagnosis of these conditions. Several workers have given directions for making the diagnosis.[436]

Abnormalities of the blood vessels are also relatively frequent and poorly understood. The inferior vena cava and other retroperitoneal veins are subject to much variation, as is to be expected in view of the complex pattern of the embryonic veins from which they arise.[437] Hereditary anomalies of these veins are on record.[438] The relationship of vascular abnormalities to malformations of the organs supplied has led to much speculation, particularly in the case of dystopic kidneys and the anomalous origin of their vessels. The old theory that abnormal renal vessels cause dystopia of the kidney is now generally abandoned.[439] It is known that the permanent renal vessels develop only after the kidneys have reached their final location and in accordance with that location.

In early chick embryos abnormalities of the extraembryonic vessels may be essential parts of complex syndromes of disturbances. Cairns [46] has described in detail the inadequate development of the yolk sac vessels in homozygous Creeper embryos and the deleterious effect on the embryo. Another abnormality, the so-called lethal ring, occurs in the wall of the yolk sac in riboflavin deficiency.[141] Excessive dilatation of intraembryonic vessels is found as a sequel of anoxia in the chick.[202]

435. Blalock, A.: Bull. New York Acad. Med. **22**:57, 1946.

436. Sussman, M. L.; Grishman, A., and Steinberg, M. F.: Am. J. Dis. Child. **65**:922, 1943. White, P. D.: Heart Disease, New York, The Macmillan Company, 1944.

437. McClure, C. F. W., and Huntington, G. S.: Mammalian Vena Cava Posterior, American Anatomical Memoir 15, Philadelphia, Wistar Institute of Anatomy and Biology, 1929, p. 1. Gruenwald, P.: Beitr. z. path. Anat. u. z. allg. Path. **101**:439, 1938.

438. McNutt, C. W., and Sawin, P. B.: Am. J. Anat. **72**:2595, 1943.

439. Gruber.[412] Gruenwald, P.: Virchows Arch. f. path. Anat. **303**:47, 1938.

Abnormalities of the blood and the blood-forming tissues may be primary, on a genetic basis, or secondary to other defects. Well studied examples of the former kind are described by Grüneberg.[1r] The latter type consists of anemia, more or less compensated by persistent extra-medullary blood formation. It occurs if there is insufficient space for marrow in the skeleton, as in chondrodystrophy,[440] or if fetal blood is destroyed by specific antibodies of the mother in so-called erythroblastosis fetalis, which will be described in more detail in a later section.

CLEFTS OF THE FACE

In older accounts the development of clefts of the face is usually attributed to the persistence of an early embryonic condition in which parts of the future face (medial and lateral nasal processes and maxillary processes) are separated from one another by fissures. Several investigators have recognized the fallacy of this explanation; particularly, Fleischmann[441] has emphasized that the so-called clefts occurring in the face of the normal early embryo are mostly grooves with a solid bottom, which disappear later on, not by fusion of adjacent parts but by filling in of mesenchyme under the intact epidermis. In the case of the groove between the medial nasal and the maxillary process the so-called cleft is filled from the beginning with epithelium of the thickened lower portion of the nasal plate, which is then partly replaced by mesenchyme.[442]

Politzer[443] shows in a critical survey that various types of abnormal cleft of the face develop in entirely different manners. Harelip, with or without corresponding clefts of deep structures, develops at the site of the aforementioned epithelial mass separating the medial nasal from the maxillary process, when mesenchyme fails to penetrate it. Early human stages of this malformation are known.[444] An oblique cleft running from a lateral part of the mouth to the eye was formerly explained as a persistent separation of the lateral nasal from the maxillary process. Politzer[443] points out that this type of cleft represents an irregular laceration of the face by mechanical force (e. g., that of an amniotic band) connecting two weak points, namely, mouth and eye. This is supported by the relation of such clefts to various structures, particularly the nasolacrimal duct, a relation which could not occur in a persistent embryonic condition. A third group, the median defects of the upper jaw, is considered by Politzer to consist of low grade cyclopic defects.

In rats cleft palate has been produced by a nutritional deficiency of the pregnant mother, which also causes a variety of other skeletal

440. Landauer, W., and Thigpen, L. W.: Folia haemat. **38**:1, 1929.
441. Fleischmann, A.: Sitzungsb. d. phys.-med. Soz. zu Erlangen **69**:315, 1937.
442. Veau, V., and Politzer, G.: Ann. d'anat. path. **13**:275, 1936.
443. Politzer, G.: Monatschr. f. Ohrenh. **71**:63, 1937.
444. Veau, V.: Ztschr. f. Anat. u. Entwcklngsgesch. **108**:459, 1938.

malformations.[445] Hereditary cleft palate of laboratory animals has been studied in several instances.[446] One instance is that in which modifying genetic factors influence the expression of the defect and may completely inhibit its appearance.[47a] Reed [47b] assumes that the inheritance observed in his strain of mice may well be similar to that in man. The genetics of human harelip and cleft palate has been discussed by Mather and Philip.[447]

TISSUE MALFORMATIONS

In this section abnormal differentiations or locations of tissues will be discussed as far as they are not part of gross anatomic malformations, and exclusive of the problem of tumor growth, which will be referred to in part III of this review. Abnormal histologic differentiation is often discussed in connection with tumor growth, partly because abnormal growth seems to produce changes in differentiation and partly because abnormal tissues often remain unrecognized unless they give rise to tumors. The older literature contains several large reviews of tissue malformations.[448]

There is no essential difference between the embryo and the adult in the developmental mechanisms of tissue malformations, and in many cases in which these have been found in adults, the time of development is unknown. For these reasons embryonic and postnatal conditions will now be treated, and no attempt will be made to find essential differences between the two kinds.

The histologic identification of abnormal tissues must be discussed here. It is often difficult and in many cases is carried out inadequately. Identifications which should be not more than suggestions are often taken for well established ones. In most cases one can demonstrate little or no evidence of a specific cellular function which might aid in the identification, and often a tissue is classified only by its appearance in histologic sections stained routinely with hematoxylin and eosin or by a similar method. Size and texture of nucleus and cytoplasm as seen in these sections are compared with those seen in normal cells. It should be clear to every one that these are but superficial and

445. Warkany, J.; Nelson, R. C., and Schraffenberger, E.: Am. J. Dis. Child. 65:882, 1943.

446. Reed and Snell.[47a] Reed, S. C.: Genetics 21:339, 1936; footnote 47 *b*. Scott.[280b]

447. Mather, K., and Philip, U.: Ann. Eugenics 10:403, 1940.

448. Meyer, R.: Ergebn. d. allg. Path. u. path. Anat. (pt. 2) 9:518, 1905; 15:430, 1911; Ztschr. f. Geburtsh. u. Gynäk. 71:221, 1912. Herxheimer, G., in Schwalbe,[256] 1913, pt. 3. Fischer-Wasels, W., in Bethe, A.; von Bergmann, G.; Embden, G., and Ellinger, A.: Handbuch der normalen und pathologischen Physiologie, Berlin, Julius Springer, 1927, vol. 14, pt. 2, p. 1211.

unreliable criteria. To give an example, tissues have been identified in many different instances as adrenal cortex on little more basis than the presence of vacuolated cytoplasm. This is justified if one finds in addition the characteristic structure of a glomerular and a fascicular zone as one sometimes does in the epididymis, or if the tissue in question is located where one would expect adrenal cortex to develop, as in close proximity of the main organ. On the other hand, a striking instance in which the diagnosis of adrenal cortex appears unwarranted to me is that of a node of large, foamy cells found in the orbit.[449] In the report of that case, neither the structure of adrenal cortex was described, nor were any special methods used which, by the demonstration of certain chemical compounds, would suggest (though not establish) the diagnosis. It is so improbable on embryologic grounds that adrenal cortex would be present in this particular location that the vacuolated appearance of the cytoplasm is insufficient for the suggested diagnosis. Similar difficulties are encountered in many other instances, particularly often in cases in which structures of the urogenital tract are involved, e.g., tumors of the gonads and the kidneys. This is probably due to the unusually wide variety of developmental potencies which the tissues of the urogenital tract possess.[450] Another field in which the identification of cells has aroused much argument is that of hemopoietic and lymphoid tissues; here, too, the cells have a great variety of potencies, at least in the early phases of their differentiation.

The range of developmental potencies plays an important role in many tissue malformations. In many instances there is no experimental proof of the multiplicity of potencies in later stages of development. However, descriptive histology and embryology have brought forward many instances in which several cell types develop from a common ancestor in late embryonic or postnatal stages. Since the possession of multiple potencies is not a peculiarity of embryonic cells, one is not justified in designating cells of the mature organism as embryonic because they appear to have latent potencies.[451] The visible differentiation of a cell type cannot serve as an indication of its range of latent potencies, and there is no reason to postulate "undifferentiated" cells when evidence of latent potencies is discovered, or to deny the possibility of activation of latent potencies because the tissue in question shows some differentiation. If one gives due consideration to the possibility of activation of normal latent potencies, the assumption of persistence of embryonic germs becomes superfluous for the explanation of most dystopic tissues.[451] There is little direct evidence of the existence of such germs.

449. Hughes, L. W., and Ambrose, A.: J. A. M. A. **126**:231, 1944.
450. Gruenwald, P.: J. Morphol. **70**:353, 1942.
451. Gruenwald, P.: Arch. Path. **36**:190, 1943.

Gruenwald [452] recognizes two principal types of development of dystopic tissues and describes examples in human embryos. One is true aberration of tissue germs; that is, they depart from the normal point of origin into abnormal locations. Examples are aberrant bile ducts and accessory adrenal medulla. The other type arises by abnormal differentiation of cells in loco; these cells do not migrate and are not displaced from the region where the tissue which they form is normally found. Examples are ectopic renal tubules, accessory adrenal cortex or bone developing in abnormal locations. These formations develop by activation of potencies which the cells in loco normally have. Induction may have a part in this activation; thus, ectopic renal tubules may under certain conditions be induced by nerve tissue [453] and bone by mucosa of urinary passages.[454]

To these mechanisms which produce tissues where they would not normally occur at any time, one might add inhibition of development, occurring either as a failure to differentiate fully or as a persistence of tissues which normally disappear. Finally, there may be devious differentiation not corresponding to any normal tissue at any stage. The occurrence of pure failure of differentiation (the undifferentiated tissue being present) is doubtful unless one wishes to regard as such the absence of a kidney in a case in which the nephrogenic tissue is present in its mesenchymal state but fails to differentiate into nephrons. However, incomplete differentiation occurs as part of a complex disturbance, such as inhibited myelination of nerve fibers in mongoloid deficiency.[444b] Failure of a tissue to disappear is responsible, for example, for ectopic thyroid tissue and epithelial ducts at the site of the embryonic thyroglossal duct or for persistence of mesenchyme in the vitreous body of the eye.[455] Devious differentiation offers great difficulties to interpretation, as the customary comparison with normal tissue fails. It occurs to some extent in many tumors, particularly if the mother tissue has an intricate structural pattern which the tumor cannot duplicate. A good example of devious differentiation is that of the grotesque cells found in the brain in tuberous sclerosis.

Of great theoretic interest are the multiple hamartioses [318] affecting, for instance, in the case of the tuberous sclerosis complex the brain, the eyes, the heart, the kidneys and the skin. Nothing is known of their cause except that they may be familial. To explain them all simply by defective organizer action, as Moolten [318] does, is not justi-

452. Gruenwald, P.: J. Urol. 48:224, 1942.

453. Gruenwald, P.: Anat. Rec. 86:321, 1943.

454. Huggins, C. B.: Arch. Surg. 22:377, 1931. Huggins, C. B.; McCarroll, H. R., and Blocksom, B. H.: ibid. 32:915, 1936.

455. Reese, A. B., and Payne, F.: Am. J. Ophth. 29:1, 1946.

fied until many other possibilities are excluded and the organizers involved are better known.

The problem of dystopic adrenal cortex and related tumors has been studied extensively. They will be discussed here as examples of tissue malformation. Many writers have believed that accessory corticoadrenal nodules developed by aberration of some of the buds which grow into the retroperitoneal tissue from the mesothelium in the early phases of adrenal development. More recently Uotila [456] and Gruenwald [451] failed to find the ingrowth of distinct cell cords during normal development. The adrenal cortex is nonepithelial for a considerable period after its origin; it develops from mesenchymal cells of the celomic wall by differentiation in loco.[451] A similar mode of origin is postulated for accessory cortical tissue in any location. All definitely identified accessory nodules appear where the mesenchyma may reasonably be assumed to possess the necessary developmental potency, namely, in the retroperitoneal tissues and their derivatives. Investigators do not know what determines the activation of this potency in some cells and not in others of these tissues. Only in an isolated instance has a condition been observed in which cortical cells regularly developed outside the adrenal gland: After complete adrenalectomy adrenal cortex tissue differentiates in the ovaries of ground squirrels and mice and maintains life indefinitely.[457] This differentiation is apparently mediated by the hypophysis, as it does not occur in hypophysectomized animals.[457a]

It is not easy to determine the extent to which accessory cortical nodules should be considered as normal. They have been described as regular components of the epididymis and the epoophoron of the rabbit.[458] In the hedgehog and the cat the testis and the ovary commonly contain them.[459] In man, accessory nodules can be found in and near the adrenal capsule on careful search in almost every adrenal gland examined, particularly in those of young persons. They are encountered in the epididymis much more commonly than the occasional case reports [460] would make one believe. A genetic influence on the amount of accessory adrenal cortex that develops was found in rats when it became apparent that certain strains are unsuitable for experi-

456. Uotila, U. U.: Anat. Rec. **76**:183, 1940.

457. Groat, R. A.: (*a*) Endocrinology **32**:488, 1943; (*b*) Anat. Rec. **89**:33, 1944. (*c*) Hill, R. T.: ibid. **94**:470, 1946.

458. Lacassagne, A., and Nyka, W.: Compt. rend. Soc. de biol. **118**:1406, 1935; **121**:95, 1936.

459. Watzka, M.: Ztschr. f. mikr.-anat. Forsch. **43**:235, 1938.

460. Freeman, A.: Arch. Path. **39**:336, 1945.

mental adrenalectomy because of the frequent occurrence of accessory tissue.[461]

The origin of the so-called hypernephroma (in the widest sense) has been controversial because this renal tumor presents structural traits resembling those of adrenal cortex. The followers of the theory of adrenal origin of the hypernephroma have referred to the frequency with which adrenal nodules occur in the kidney, sometimes combined with hypernephroma.[462] Cases in which adrenal gland and kidney are enclosed in a common capsule have also been cited in this connection.[463] Furthermore, in the tumors in question the distribution of vitamin A resembles that in the adrenal cortex.[464'] Actually, the adrenal tissue occurring in the kidney is of significance not so much to account for the point of origin of the tumor as to indicate that the tissue in which these cell groups have developed has adrenal cortical potencies. The presence of these potencies would make it possible for renal tissue to form an adrenal tumor or a growth of hybrid differentiation combining traits of kidney and adrenal tissue.[465] The fact that tubules are present in some of these tumors does not exclude a relationship to adrenal cortex, since lumens occur in corticoadrenal cell cords in mammals,[466] as well as in human embryos and infants.[467]

Dystopic endometrium can probably develop in several manners. When 'it occurs in the myometrium as adenomyosis, it has obviously appeared there by growing in from its normal location. On the other hand, endometriosis outside the uterus must be explained either by implantation of endometrial germs or by activation of latent potencies. It has been demonstrated that all known locations of endometriosis are such that the presence of the necessary potency is a reasonable assumption.[468]

The cutaneous nevus has received much attention during recent years, and most observers now agree that it contains nervous elements

461. Gaunt, R.: Am. J. Physiol. **103**:494, 1933. Gaunt, R.; Gaunt, J. H., and Tobin, C. E.: Proc. Soc. Exper. Biol. & Med. **32**:888, 1935. Cleghorn, R. A.; Cleghorn, S. M. M.; Forster, M. G., and McVicar, G. A.: J. Physiol. **86**:299, 1936. Ingle, D. J., in The Rat in Laboratory Investigation, Philadelphia, J. B. Lippincott Company, 1942, p. 291.

462. Mitchell, N., and Angrist, A.: Arch. Path. **35**:46, 1943.

463. O'Clowley, C. R., and Martland, H. S.: J. Urol. **50**:756, 1943.

464. Popper, H., and Ragins, A. B.: Arch. Path. **32**:258, 1941.

465. Schiller, W.: Arch. Path. **33**:879, 1942. Gruenwald.[452]

466. da Costa, A. C.: Compt. rend. Assoc. anat. **23**:69, 1928.

467. Hett, J.: Ztschr. f. mikr.-anat. Forsch. **3**:179, 1925.

468. Gruenwald, P.: Am. J. Obst. & Gynec. **44**:470, 1942.

and distorted tactile corpuscles.[469] This conclusion has distracted attention from the old argument as to whether the nevus tissue originates from the epidermis or the connective tissue.. Recent workers have not discussed the problem of the origin of the nevus cell. It seems to me that the relation to nerve tissue, the pigmentation and the presence in the skin and (as melanoma) in the meninges are satisfactorily accounted for by assuming an origin from neural crest cells. These cells are known to migrate in the vicinity of the central nervous system and also along the skin of the embryo. They form either nerve cells and their supporting cells (connective tissues)[470] or the pigment cells of the skin.[471] Foot [472] does not mention this obvious possibility in a chart of the normal tissues and tumors derived from the neural crest.

A special problem apart from others in this field is that of teratomas. It is obvious that teratomas containing a variety of tissues must originate from pluripotent, if not omnipotent, cells, which thus resemble either germ cells or blastomeres or the cells in a few areas of the embryo where germ layers are not distinct and one cell group gives rise to a great variety of tissues (e.g., the prechordal region or the primitive streak). All of these possibilities have been suggested as the germs of teratoma. The problem has been reviewed by Schwalbe.[256] More recently Holmdahl [473] has accepted all of the aforementioned possible sources but favors the origin from areas where no distinct germ layers differentiate. Schlumberger [474] assumes that teratomas of the gonads originate from primordial germ cells and that teratomas arising in other places are due to "dislocation of tissues during embryogenesis," particularly in his cases of teratoma of the anterior mediastinum. In support of the theory of origin from germ cells, reference has been made to the parthenogenesis-like development of egg cells observed in the ovaries of various mammals.[475] Michalowsky and

469. Masson, P.: Ann. d'anat. path. 3:417, 657, 1926. Ewing, J.: Brit. M. J. 2:852, 1930. Foot, N. C.: Am. J. Path. 8:309 and 321, 1932. Laidlaw, G. F., and Murray, M. R.: ibid. 9:827, 1933. Becker, S. W.: Arch. f. Dermat. u. Syph. 30:779, 1934. Feyrter, F.: Virchows Arch. f. path. Anat. 301:417, 1938. Montgomery, H., and Kernohan, J. W.: J. Invest. Dermat. 3:465, 1940. Ramel, E.: Schweiz. med. Wchnschr. 71:375, 1941. Roth, G.: Arch. f. Dermat. u. Syph. 183:148, 1942.

470. Raven, C. P.: Arch. f. Entwcklngsmechn. d. Organ. 129:179, 1933.

471. DuShane, G. P.: Quart. Rev. Biol. 18:109, 1943; 19:98, 1944.

472. Foot, N. C.: Pathology in Surgery, Philadelphia, J. B. Lippincott Company, 1945.

473. Holmdahl, D. E.: Acta path. et microbiol. Scandinav. 19:603, 1942.

474. Schlumberger, G. H.: Arch. Path. 41:398, 1946.

475. Loeb, L.: Arch. f. mikr. Anat. 65:728, 1905; J. A. M. A. 56:1327, 1911. Courrier and Oberling: Bull. Soc. anat. de Paris 93:724, 1923. Kampmaier, O. F.: Am. J. Anat. 43:45, 1929.

others [476] have produced teratomas in the testes of fowl by injecting zinc salts. It has recently been suggested that many of the common types of testicular tumors of man are teratomas or resemble teratomas in that they are "neoplastic expressions of the unlimited potencies of embryonic cells." [477] Many of these tumors contain trophoblastic tissue.

Budde [478] compares the origin of teratomas with that of double monsters. If the malformation originates early in the development of an embryo, it results in a double monster, and if a comparable disturbance occurs later, it produces a teratoma. This matches well with the observation of Edmonds and Hawkins [259] that twins and teratomas occur in the same families. The problem of the homology of teratoma with an embryo (i.e., a parasitic twin) has been discussed by many writers. Nicholson,[479] Willis [480] and Needham [4] deny any homology of the two. One of their principal arguments is based on the lack of organization of the whole structure as indicated by the absence of segmentation. However, Schauffler [481] reports a teratoma with a segmented vertebral column. Other cases of a fetiform inclusion with extremities and other fairly well organized parts of a body have recently been reviewed, and a new case added by Plaut.[482] He considers the (so far not observed) development of the highly organized malformation, with skull, vertebrae, extremities and other parts, as a distorted replica of the development of an embryo. It is true, as Needham [4] states, that many times "innominate" structures in teratomas have unjustifiedly been compared to complex organs; yet there are well authenticated instances of high differentiation (e.g., of liver and kidney [479] or cerebellum [483] in addition to the aforementioned fetiform inclusions.

In recent years interest has shifted from the mother tissue of teratoma to the mechanism of development. In this connection organizer phenomena have been widely mentioned as responsible for the structure of the teratoma. Krafka [484] assumes in his organizer theory the pro-

476. Michalowsky, I.: Centralbl. f. allg. Path. u. path. Anat. **38**:585, 1926. Bagg, H. J.: Am. J. Cancer **26**:69, 1936. Anissimova, V.: ibid. **36**:229, 1939. Falin, L. I., and Gromzewa, K. E.: ibid. **36**:223, 1939; Virchows Arch. f. path. Anat. **306**:300, 578, 1940.

477. Friedman, N. B., and Moore, R. A.: Mil. Surgeon **99**:573, 1946.

478. Budde, M.: Beitr. z. path. Anat. u. z. allg. Path. **75**:357, 1926.

479. Nicholson, J. W. D.: J. Path. & Bact. **32**:365, 1929.

480. Willis, R. A.: J. Path. & Bact. **40**:1, 1935; **45**:49, 1937.

481. Schauffler, G. C.: Pediatric Gynecology, Chicago, The Year Book Publishers, Inc., 1942.

482. Plaut, A.: J. Mt. Sinai Hosp. **12**:567, 1945.

483. Willis, R. A.: J. Path. & Bact. **49**:571, 1939.

484. Krafka, J., Jr.: Arch. Path. **21**:756, 1936.

duction of secondary embryonic axes by "interference with the normal effect of the organizer," in analogy with the secondary body axes induced in amphibians by implantation of additional organizers. In essence, this theory comes close to that of Budde [478] (see the foregoing paragraph) and to the theory of blastolysis of Werber [101b] (see part I). The presence of organizer action within organ primordia in teratomas, e.g., kidneys, is obvious.

Needham [4] distinguishes two phases of organizer action: evocation and individuation. Evocation stimulates differentiation but does not determine its exact pattern, whereas individuation includes the determination of complex and harmonious developmental patterns. In the ectoderm of experimental embryos the induction of nervous tissue produces under certain conditions irregular tubes or cell masses by evocation, and under other conditions a neural tube with brain vesicles at one end, which is individuation. If a piece of tissue which would have the latter effect when transplanted in the living state is boiled before implantation, it will still act as evocator but will no longer produce individuation. Needham therefore compares teratomas with their irregular arrangement of highly differentiated tissues to the effects of dead organizers (without implying that there was actually induction on the part of dead tissue). Since, however, some teratomas show a considerable measure of individuation (see a foregoing paragraph), it might be better to assume that there is loss of individuation in the development of teratomas to a varying, though usually high, degree. Needham further points out that evocators occur commonly in the organism, and it may therefore be more important to give consideration to the presence of cells which will react to these evocators by producing the variety of structures found in teratomas. This leads back to the older embryologic speculations about tissues which may be expected to have a sufficient range of developmental potencies (blastomeres, germ cells and others).

EXAMPLES OF SYNDROMES OF MALFORMATIONS

A few well studied syndromes will be described, which affect several parts of the body. Each of these has been referred to in the foregoing pages, and it remains to summarize and correlate the various abnormalities.

The Creeper Fowl.—The Creeper gene (Cp) is dominant over its normal allelomorph, or allele (cp), and produces in the heterozygous condition chondrodystrophy. The gene is lethal, and most CpCp embryos die on the fourth to sixth day of incubation. A few survive but fail to hatch; these have phocomelia and ocular malformations. In early stages Cpcp embryos show no abnormalities and cannot be

distinguished from normal (cpcp) ones. Among the CpCp embryos there is considerable variation of structure, and only recently satis-factory criteria have been established.[485] All of these embryos have a defective yolk sac circulation. If this is severe and the vitelline vessels do not develop as continuous channels at all, the embryos show severe general retardation, intraembryonic anastomoses between large arteries and veins, and asymmetry of the eyes and the otocysts.[46] This asymmetry exceeds the extent found in normal embryos and is explained by the fact that the oxygenation afforded the organs by the blood stream is deficient; the part near the egg shell (in normally rotated embryos, the right side of the head) gets a better supply of oxygen by diffusion than do the parts on the opposite side.[46] In those CpCp embryos in which a better vitelline circulation develops, the effects on the embryo are at first minimal or absent (these embryos have not been examined in serial sections). The vascular network of the yolk sac is abnormal in these as well; there is no terminal vein, and part of the vessels contain stagnant blood. Eventually all circula-tion in the vitelline vessels stops, while the heart keeps pulsating, and the embryo dies shortly thereafter.[485] Just what the early characteristics of those CpCp embryos are which live longer and become phocomelic is not known. Landauer[51] recently reported that a higher proportion of CpCp embryos survive to late periods of incubation if the tempera-ture during the first day of incubation is kept lower than usual, at 96 F.

The developmental potencies of CpCp and Cpcp limb buds have been studied by explantation.[389a] In the limb bud stage the phocomelic and chondrodystrophic abnormality appears to be determined, since transplanted limbs develop as they would in the embryo from which they came (apart from changes which the altered conditions would produce in any limb). When the effect of the disturbed circulation in CpCp embryos was recognized, regions containing the limb material were transplanted from embryos prior to the development of blood vessels. These also developed into phocomelic or chondrodystrophic structures, depending on the genotype. Apparently abnormal circula-tion has no part in the determination of phocomelia.[389b]

Experimental transplantation of the eyes of CpCp embryos has been reviewed in a foregoing section. The experiments show that the coloboma of the phocomelic embryo is also independent of the abnormal circulation. In contrast to the skeletal changes, however, it is not inherent in the primordium of the eye at the time of transplantation. It seems that an influence of some adjacent part determines this malformation.[337] In a review of the manifestations of the homozygous Creeper condition which incorporates part of the work just quoted,

---

485. Cairns, J. M., and Gayer, K.: J. Exper. Zool. **92**:229, 1943.

Hamburger [19] finds four abnormal mechanisms which are not correlated by any developmental links known to investigators. (1) In the majority of CpCp embryos a deficiency of the vitelline circulation leads to various severe malformations and early death; a mild degree of this may exist in those embryos which survive to become phocomelic, and may exert a hitherto unknown influence. (2) A disturbance of chondrogenesis combined with (3) a reduction of growth of the limbs produces phocomelia. There is an insufficient amount of bone marrow in this condition, and this leads to anemia and enlargement of the heart and the spleen. (4) An abnormality of the head mesenchyme apparently induces ocular malformation in phocomelic emybryos. (The asymmetric malformations of the eyes of early prothanic CpCp embryos are due to the first-mentioned mechanism.)

As to the chondrodystrophy of Cpcp chicks, it will be remembered that Landauer [385c] holds that it differs from phocomelia only in degree. Ocular malformations do not occur in these chicks.

"Myelencephalic Blebs" in Mice.—Much of the interest in this hereditary trait has been directed toward the production of the mutation, which is supposed to have been caused by roentgen irradiation of an animal from which this stock was derived. Some investigators believe now that there was no causal relationship between the irradiation and the origin of the mutation (see part I). The genetics of this trait has been extensively studied.[19]

The malformations are partial defects of the jaws, the eyes, the extremities and possibly the kidneys.[381] There is considerable variability of expression, and only part of the defects are present in a single animal. Bonnevie [334] found that the initial abnormality consists in the discharging of an excessive amount of cerebrospinal fluid from the fourth ventricle through the normal foramen anterius into the overlying tissue. This fluid forms blebs between the epidermis and the cutis. Owing apparently to peculiarities of the shape of the body, these blebs move in the same layer until they are either resorbed or reach points from which they cannot escape, such as the tips of the jaws or the extremities or the eyes. The temporary presence of blebs causes no permanent changes. However, when they come to rest, hemorrhage occurs into their cavities, and eventually they produce a local disturbance of development in the regions mentioned.

When this trait was crossed out to different stocks of mice, the effects varied somewhat, so that the existence of genic modifiers had to be assumed. Bonnevie [334] suggests that these modifiers act by changing the curvatures of the embryonic body which, in turn, influences the direction in which the blebs move.

Brown [411] described in detail the development of the renal malformations of the same strain of mice. They consist in failure of the

ureteric bud to grow out to its full length or to branch properly and in associated failure of the metanephric blastema to differentiate into nephrons. These events are obviously not determined by the presence of blebs of cerebrospinal fluid. Bonnevie[334] failed to find any malformations of the kidneys in her mice and concluded that these abnormalities are probably not effects of the same gene which is responsible for the blebs. Another possible explanation for this discrepancy is the action of modifiers.

Bonnevie's account of the origin of the malformations of this strain has furnished what is perhaps the best known example of a mechanical correlation of multiple defects of various parts of the body. Unfortunately, this has stimulated unwarranted speculation and has led several writers to extend a theory of myelencephalic blebs to totally unrelated conditions. Ullrich[486] quotes as evidence of the validity of this theory in human teratology a description of a 4 month fetus with cystic dilatation of lymph spaces in the neck.[487] Both the stage of development and the nature and the location of the cysts in that case are entirely incompatible with myelencephalic blebs. Engel[488] explains by this bleb theory a dozen unrelated syndromes of malformations, among them mongoloid deficiency and gargoylism (lipochondrodystrophy). Fortunately, these theories have not received undue consideration by other authors.

Effects of Heterospecific Pregnancy.—Certain differences of blood groups between mother and fetus cause various, often fatal disturbances in the latter if antibodies against the group-specific substance of the fetus develop in the mother.[62] The best studied effect is the so-called erythroblastosis fetalis, caused by destruction of fetal erythrocytes by maternal antibodies. In about 90 per cent of the cases this is found in Rh-positive babies of Rh-negative mothers (i.e., the baby's erythrocytes are shown by test to contain an Rh factor, while the mother's are shown not to contain it); in the remaining instances there are other group differences, namely, of the A or B groups.[63] The complex genetics of the Rh-types has been studied by Wiener, Sonn and Polivka.[489] Newborn babies with this disease have enlargment of the liver and the spleen and often also of the adrenal glands, the heart and the pancreatic islets.[140b] Similar enlargements occur in children of diabetic mothers.[140b] and also occasionally without any known cause. The

486. Ullrich, O.: Klin. Wchnschr. **17**:185, 1938.
487. Gruenwald, P., and Kornfeld, W.: Beitr. z. path. Anat. u. z. allg. Path. **96**:341, 1936.
488. Engel, D.: Am. J. Dis. Child. **60**:562, 1940.
489. Wiener, A. S.: Tr. & Stud., Coll. Physicians, Philadelphia, **13**:105, 1945. Wiener, A. S.; Sonn, E. B., and Polivka, H. R.: Proc. Soc. Exper. Biol. & Med. **61**:382, 1946.

mechanism of these changes is unknown. Histologically, extensive extramedullary blood formation is found in various organs in fetal erythroblastosis. However, this does not fully account for the gross changes just mentioned. Macklin [490] suggests that erythroblastosis be diagnosed only if iron deposits due to hemolysis are demonstrable in the liver by histochemical means.

Changes other than anemia and its immediate sequelae have been produced by heterospecific pregnancy. They are of great importance and perhaps not fully recognized at the present time. Universal hydrops of the fetus is a well known manifestation.[322] It occurs occasionally without apparent relation to heterospecific pregnancy.[491] A reaction between antigen and antibody occurring in the body cells has been suggested as the cause of the damage of the liver, since the jaundice in some cases is out of proportion to the hemolysis.[492] The damage of the brain may be due to the damage of the liver,[322d] to the anemia [322c] or to agglutination thrombi lodged in cerebral blood vessels.[493] If it occurs in jaundiced patients, it is often quite conspicuous as the so-called kernicterus (encephalomyopathy with icterus). The presumable sequelae of this condition have been reviewed by Docter.[322f] In a small number of cases kernicterus is not associated with erythroblastosis.[494] There are indications that damage of the brain due to heterospecific pregnancy may occur without other manifestations, remain unnoticed in the young infant and account for a considerable percentage of cases of undifferentiated feeblemindedness.[322] Levine [495] suggests that fetal or neonatal death without characteristic anatomic findings of hydrops or erythroblastosis may also be due to the same cause. Haldane [496] estimates that the effects of Rh-group differences between mother and fetus account for more human deaths than any other genic difference.

Wiener [497] has recently stated that a difference of action between two kinds of antibodies which the mother may form accounts for two different syndromes within the group of erythroblastosis (in the wider sense). Agglutinins are believed to cause fetal erythroblastosis (in the

490. Macklin, M. T.: J. Pediat. **25**:533, 1944.

491. Potter, E. L.: Am. J. Obst. & Gynec. **46**:130, 1943.

492. Gilmour.[322d] Leonard, M. F.: J. Pediat. **27**:249, 1945.

493. Wiener, A. S., and Brody, M.: Science **103**:570, 1946; Am. J. Ment. Deficiency **51**:1, 1946.

494. Forster, F. M., and McCormack, R. A.: J. Neuropath. & Exper. Neurol. **3**:379, 1944.

495. Levine, P.: J. Hered. **34**:71, 1943.

496. Haldane, J. B. S.: Ann. Eugenics **11**:332, 1942.

497. Wiener, A. S.: Am. J. Dis. Child. **71**:14, 1946; New York State J. Med. **46**:912, 1946; Proc. Soc. Exper. Biol. & Med. **61**:390, 1946.

strict sense), jaundice of the newborn (icterus gravis neonatorum) and encephalomyopathy with icterus (kernicterus)'. The agglutination thrombosis of blood vessels of various organs which Wiener holds responsible for damage of tissues, has not been found ·by other workers up to this time. If, on the other hand, blocking antibodies pass from the mother to the fetus, severe sequelae will include death of the fetus, which in many instances shows hydrops; in less severe involvement there will be congenital hemolytic anemia. The. ability of the mother to produce one or the other kind of antibody against the fetal Rh antigen depends on a hereditary factor.

The knowledge of heterospecific pregnancy has already led to the establishment of diagnostic methods and to rational directives for transfusion therapy of the hemolytic anemia. However, improved postnatal treatment will prevent death from anemia only. Other, perhaps more serious damage, which occurred before birth, cannot be undone. Investigators have not as yet established the full range of abnormal conditions caused by heterospecific pregnancy, particularly those in the field of neuropsychiatry. It may turn out that many of the infants who are saved by better postnatal care have severe defects unless, as Wiener [497] has suggested, those infants who can be saved are not the ones with damage of the brain.

(To Be Concluded)

Notes and News

Appointments, Etc.—A. Learner, Oak Ridge Hospital, Oak Ridge, Tenn., is now pathologist at the Norwegian-American Hospital, Chicago, succeeding H. R. Fishback.

In the college of medicine of the University of Nebraska, Omaha, John R. Schenken has been promoted from associate professor to professor of pathology and acting chairman of the department; J. P. Tollman has been appointed chairman of the department of clinical pathology and bacteriology; Pliney Allen has been appointed assistant professor of pathology and pathologist at the Immanuel Deaconess Institute, Omaha.

Since his retirement as head of the department of pathology and bacteriology in the University of Nebraska College of Medicine, H. E. Eggers has been named director of the cancer control division of the state health department.

Israel Davidsohn has resigned as associate professor of pathology in the University of Illinois College of Medicine and has accepted appointment as professor of pathology and head of the department in the Chicago Medical School.

F. W. Sunderman has been appointed professor of clinical pathology in Temple University School of Medicine, Philadelphia.

Awards.—The Nobel Prize in Medicine has been divided between Bernardo A. Houssay, Buenos Aires, Argentina, for his "discovery of the significance of the hormone produced by the frontal lobe of the hypophysis," and Carl F. and Gerty T. Cori, Washington University, St. Louis, for their discovery of the "mechanism of enzymatic synthesis of glycogen or animal starch" through the isolation of phosphorylase.

Kendall Emerson, New York, director of the National Tuberculosis Association, has received the 1947 Trudeau Medal for meritorious service in the campaign against tuberculosis.

Awards of the Albert and Mary Lasker Foundation for 1947 have been given to: Thomas Parran, surgeon general, United States Public Health Service, as a special award for the work he has done toward the control of venereal disease; O. T. Avery, of the Rockefeller Institute for Medical Research, for studies on the antigenic constitution of bacteria; to Thomas Francis Jr., of the University of Michigan, for his contribution to the knowledge of influenza; to H. W. Smith, New York University College of Medicine, for studies in cardiovascular and renal physiology, and to Alice Hamilton, Hadlyme, Conn., for her services in the prevention of occupational diseases.

Society News. — At the annual meeting of the Oklahoma Association of Pathologists, Béla Halpert was elected president and Emil Patek vice president. J. N. Owens Jr., Shawnee, is the secretary-treasurer.

The National Society for Medical Research was organized in 1946 by the medical colleges, research hospitals and other institutions interested in biology, medicine, dentistry, pharmacy and veterinary medicine, to promote a better public understanding of the principles and methods of scientific investigation. The president is A J. Carlson, the secretary-treasurer A. C. Ivy, and the executive secretary R. A. Rohweder (25 East Washington Street, Chicago 2).

At the twenty-sixth annual meeting, the American Society of Clinical Pathologists inducted T. J. Curphey as president and O. A. Brines was made president-elect. The Ward Burdick Award was given to Charles Sheard for his work in photelometry and spectrophotometry.

Books Received

Le Granulomatosi Fungine Dell'uomo Nelle Regione Tropicali e Sub-tropicali. By Piero Redaelli, director of the Institute of Anatomy and Pathologic Histology of the University of Milan, and Raffaele Ciferri, director of the Botanical Institute, Faculty of Agriculture and Forestry, of the University of Florence. Pp. 698, with 195 illustrations. Price 4,000 lires. Florence, Italy: S. E. S., 1942.

The names of Redaelli and Ciferri attached to a book on mycoses is enough to arouse great expectations. In the introduction the authors make honorable mention of twenty-three predecessors of the present time who have undertaken the difficult task of dealing in a systemic fashion with fungi and fungi-produced diseases. It must be recognized that this most congenial association of a pathologist (Redaelli) and a botanist (Ciferri), each an authority in his field, has resulted in a monumental work which surpasses many of the previous efforts of others, being complete and thorough both from the mycologic and from the medical standpoint. As such, the book represents an excellent addition both to the library of the botanist and to that of the physician.

The 698 pages of the book are divided into 16 clear, concise and beautifully illustrated chapters. The first four chapters of the general part are devoted to the classification of the fungi, to their geographic distribution and to problems of climate, and they offer an excellent understanding of the immunobiologic and morphologic aspects of the development of the mycoses.

Each of the 12 chapters of the special part deals with its topic systematically and easily carries the reader from the early, well documented historical observations to the most modern views on classification, etiologic factors, morphologic aspects and treatment. The system adopted is rigidly applied to each chapter, resulting in an exhaustive study of the granulomatoses due to Actinomyces, Rhinosporidium, Coccidioides, Paracoccidioides, Sporotrichum, Blastomyces and other fungi.

Of particular interest are the chapters on "Darling histoplasmosis" as a systemic disease of the reticuloendothelial system and on hyphomycosis, hemisporosis and oosporosis, mainly based on the wide experience of the authors.

An impartial, well assembled bibliography is attached to each chapter.

Both the writers and the publisher are to be congratulated on this magnificent volume, a translation of which would certainly represent a valuable addition to the American literature on mycoses.

Encyclopedia of Endocrinology. By Hans Selye, M.D., Ph.D. (Prague), D.Sc. (McGill), F.R.S. (Canada), professor and director of the Institute of Experimental Medicine and Surgery, University of Montreal. Section IV, "The Ovary": Volume VII, "Ovarian Tumors" (pp. 349 and 38 plates), and Bibliography (pp. 427). Price $21.75. Montreal: Richardson, Bond and Wright, 1946.

This book is in two parts, of which one is loose leaf and the other permanently bound. The former contains 289 pages of text with detailed descriptions of ovarian tumors, a subject index and numerous photographs, photomicrographs and drawings. It also contains a 60 page list of periodicals. The second part is a bibliography of 427 pages containing references to about 17,000 papers. To eliminate the possibility of errors in typesetting, this part was printed with electromatic typewriters and subsequently reproduced by a photographic process.

The "Encyclopedia of Endocrinology" as planned will consist of ten sections. This book is part of the fourth section. It is the second to be published, having been preceded, in 1943, by section I, entitled "Classified Index of the Steroid Hormones and Related Compounds." Section IV, "The Ovary," will eventually be composed of seven volumes, of which this is the last. The purpose of the encyclopedia is ". . . to demonstrate—using endocrinology as an example—the essential feasibility of a rational classification and evaluation of all publications pertinent to a large field of medicine." The author was disturbed by the possibility that scientific production might outgrow classification and critical evaluation of the data. Having in his library 250,000 entries to subjects in endocrinology, he began the encyclopedia. The loose leaf system was used to permit easy revision, in part or in whole.

This volume is truly encyclopedic in the best sense. All types of ovarian and paraovarian tumors occurring in man and animals are discussed critically and, presumably, exhaustively as well. The writing is authoritative without being dogmatic. Every one who deals with ovarian tumors should have access to this exceptionally complete and valuable volume.

QUANTITATIVE ESTIMATION OF THE FIBROUS TISSUE IN PATHOLOGIC LIVERS

SHIELDS WARREN, M.D.

AND

P. N. WAHI, M.D.

BOSTON

SINCE neither liver function tests nor histologic studies gage accurately the extent of damage or of fibrosis of the liver, an attempt was made to establish a quantitative basis for the estimation of fibrosis as an index to the degree of permanent damage of the liver. In the development of alcoholic and some other types of cirrhosis there is initial reversible change, characterized chiefly by fatty infiltration, which is later followed by an irreversible stage, characterized chiefly by increased fibrosis. Thus the amount of fibrous tissue in the liver may be used to estimate the extent of damage and hence the prognosis in cases of cirrhosis.

In connection with studies of cirrhosis of the liver, therefore, it is desirable to obtain quantitative estimates of fibrous tissue for both normal and cirrhotic livers. The method is applicable to samples weighing as little as 0.4 Gm. and thus will be useful in determining the percentage of fibrous tissue even in pieces of liver obtained by peritoneoscopy.

MATERIAL AND METHODS

The material consisted of samples of liver tissue obtained at a series of 42 autopsies, which included 8 livers showing various stages of fibrosis and 34 noncirrhotic livers. The method for measuring collagen consists in extracting from a weighed amount of liver tissue substances other than collagen and elastin. No attempt was made to determine separately the amounts of collagen and elastin, as it seemed unnecessary for the purpose of this work. Different methods of extraction have been used by various workers.[1] In the present study the amount of fibrous tissue was determined by the method of Lowry, Gilligan and Katersky [2] as follows:

1. Schepilewsky, E.: Arch. f. Hyg. **34**:348, 1899. Mitchell, H. H.; Zimmerman, R. L., and Hamilton, T. S.: J. Biol. Chem. **7**:379, 1927. Spencer, H. C.; Morgulis, S., and Wilder, V. M.: ibid. **120**:257, 1937. Smith, E. C. B.: J. Soc. Chem. Indust. **54**:152, 1935. Hoppe-Seyler, G., and Lang, K.: Ztschr. f. physiol. Chem. **215**:193, 1935.

2. Lowry, O. H.; Gilligan, D. R., and Katersky, E. M.: J. Biol. Chem. **139**: 795, 1941.
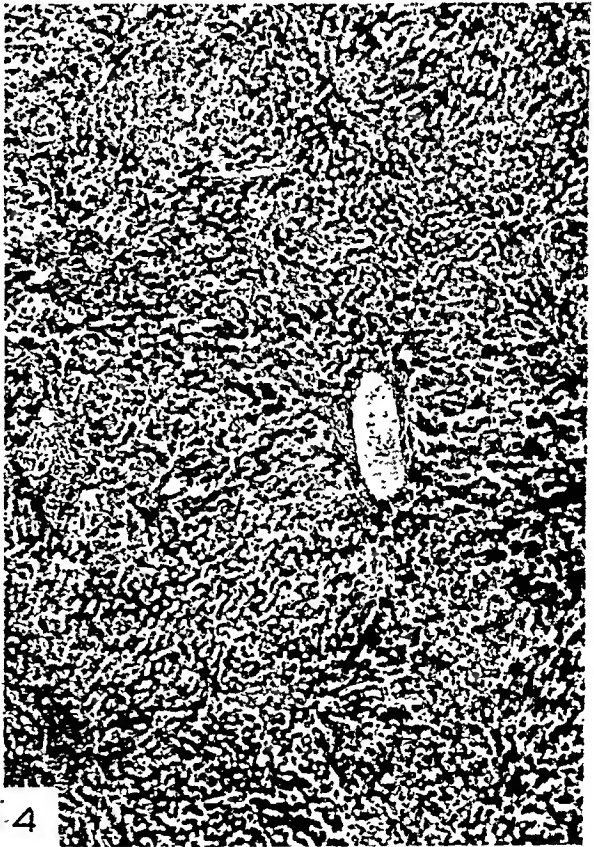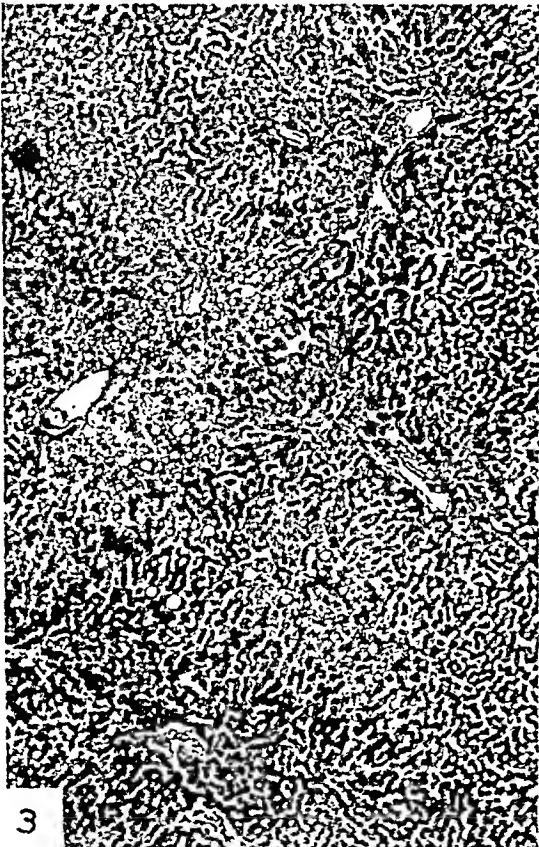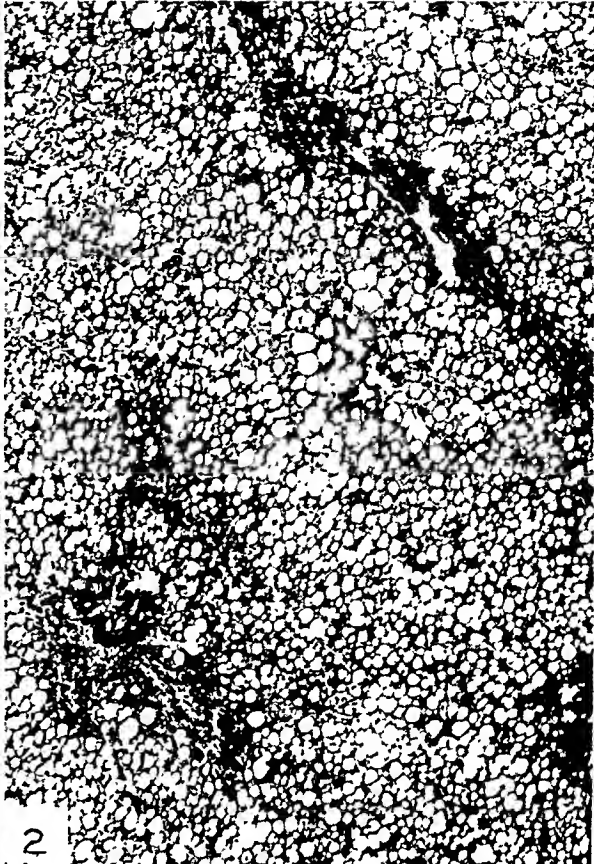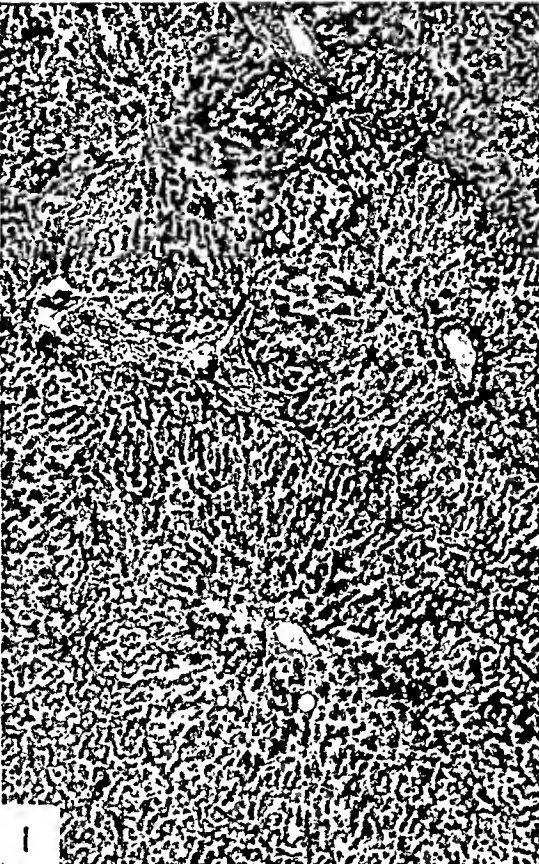
(See legends on opposite page)

A 2 Gm. sample made up of portions of liver taken from different regions was used. In cases of early cirrhosis, two different samples were taken from the right and left lobes, respectively. The tissue was finely minced and then ground in a small porcelain mortar. It was rinsed into a 50 cc. round bottom, heavy pyrex centrifuge tube with tenth-normal sodium hydroxide until the total volume was about 40 cc. The mixture was stirred and allowed to stand for twenty-four hours. It was again stirred, centrifuged and the supernatant fluid pipetted off. Again 40 cc. of tenth-normal sodium hydroxide was added; the precipitate was stirred and allowed to stand for about two hours, with occasional stirring. It was again centrifuged and. the supernatant fluid pipetted off. Forty cubic centimeters of water was then added, together with a drop of 0.1 per cent phenol red (i.e., phenol-sulfonthalein) solution. The hydrogen ion concentration was adjusted to pH 7 (faint pink color) with tenth-normal hydrochloric acid. The preparation was centrifuged, and the supernatant fluid was removed. Forty cubic centimeters of a mixture of 3 parts of 95 per cent alcohol and 1 part of ether was added and stirred. It was allowed to stand for ten minutes and then was centrifuged. The supernatant fluid was removed by suction, and 40 cc. of ether was added; the mixture was stirred and centrifuged, and the supernatant fluid was pipetted off. The outside of the tube was wiped, and the tube with the material was dried in the oven at 100 C. for about four hours, i. e., to constant weight. It was cooled to room temperature and weighed or left in a desiccator until weighed (*A*). The dried material was then fixed in Zenker's solution, embedded and stained by Masson's trichrome technic, and microscopically examined to make sure that nothing but collagenous tissue had remained behind. The tube was then cleaned, dried and weighed (*B*).

Calculation: $\dfrac{A-B}{2} \times 100 =$ percentage of collagenous substances in the tissue.

Duplicate extraction was done in each case, and the mean of the two results was taken. Samples of liver tissue weighing 0.4 Gm. were used in some cases of cirrhosis with a fair amount of accuracy. As already pointed out, this procedure might be useful in determining the percentage of fibrous tissue in pieces of liver obtained by peritoneoscopy.

The result in each case was checked by a histologic study of the dried extracted tissue, to see if all the parenchyma and infiltrating cells, if any, had been dissolved out. Tissues were fixed in Zenker's solution and stained by Masson's trichrome technic, hematoxylin-eosin and eosin-methylene blue. Stained sections were studied with a view to establishing a possible correlation between the quantitative data and the histologic observations.

EXPLANATION OF FIGURES 1 TO 4

Fig. 1.—Section of liver with 1.5 per cent fibrous tissue. Hematoxylin and eosin stain; $\times$ 50.

Fig. 2.—Section of liver showing extensive fatty infiltration. The fibrous tissue content was 2 per cent. Hematoxylin and eosin stain; $\times$ 50.

Fig. 3.—Section of liver with 2.5 per cent fibrous tissue. Hematoxylin and eosin stain; $\times$ 50.

Fig. 4.—Section of liver with 2.8 per cent fibrous tissue. Hematoxylin and eosin stain; $\times$ 50.

(See legends on opposite page)
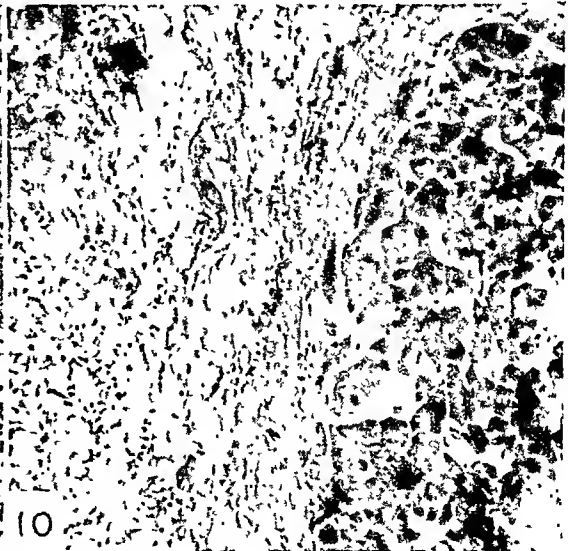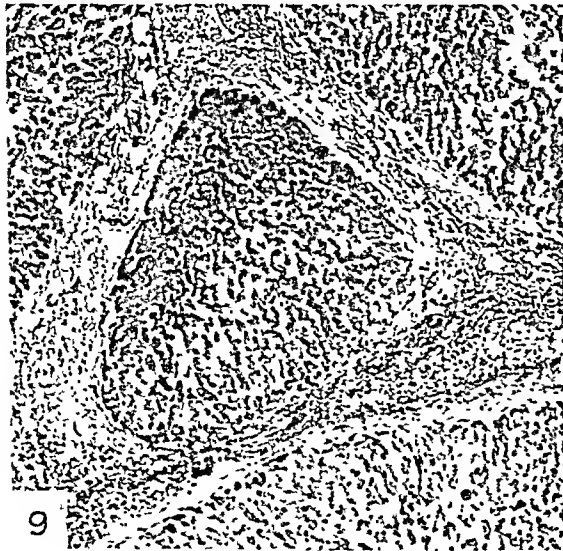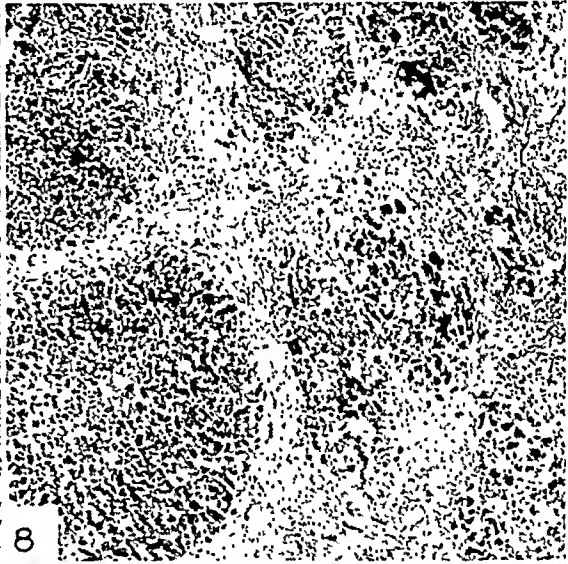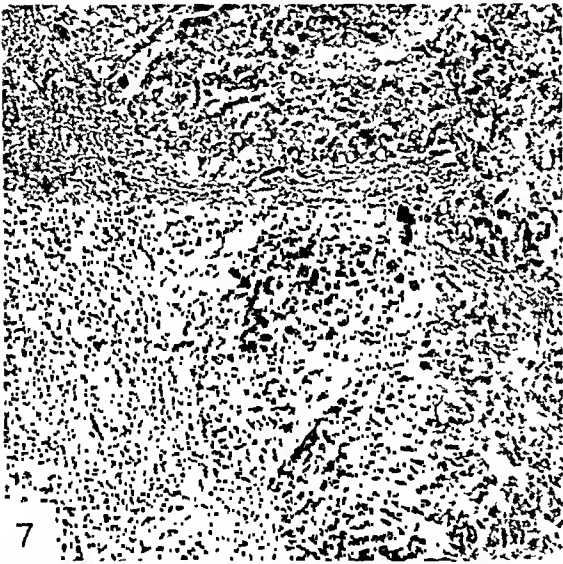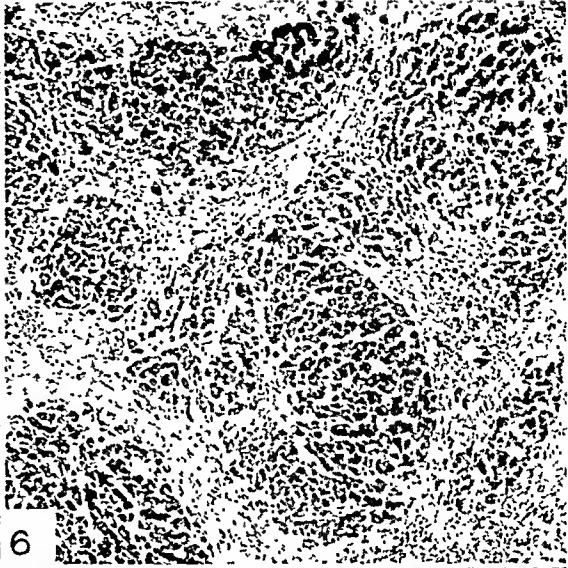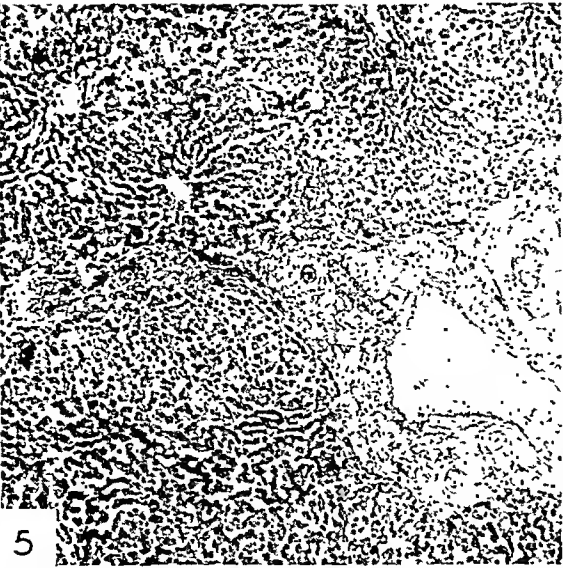
RESULTS

Fibrous Tissue Contents of Fibrotic Human Livers.—Samples of 8 human livers showing fibrosis were analyzed for fibrous tissue content. These livers included all grades of increase of fibrous tissue, from early to fully developed cirrhosis.

The table gives the percentages of fibrous tissue found for livers that were fibrotic as a result of various pathologic processes.

It is evident from the study of photomicrographs of livers showing different amounts of fibrous tissue that in the lower range of fibrous tissue content there is a fairly good correlation between the amount determined quantitatively and that seen histologically (figs. 1 to 7). With higher values it becomes difficult to judge the amount of fibrous tissue; i. e. figure 8, showing a section of liver with 13.3 per cent, and figure 9, showing one from a liver with 17.5 per cent, of fibrous tissue appear to show about the same extent of fibrosis.

Fibrous Tissue Contents of Fibrotic Human Livers

Liver	Histologic Observation	Percentage of Fibrous Tissue
1	Early portal cirrhosis	3.5
2	Portal cirrhosis	5.1
3	Portal cirrhosis	6.3
4	Portal cirrhosis	13.3
5	Portal cirrhosis	17.5
6	Healed yellow atrophy	23.0
7	Portal cirrhosis	20.2
8	Healed yellow atrophy	14.8

Fibrous Tissue Contents of Noncirrhotic Livers.—Thirty-four noncirrhotic livers comprising a wide variety of conditions but no primary or metastatic tumors were examined for their fibrous tissue content. The histologic examination did not show increases of fibrous tissue in the portal areas or in any other situation. The fibrous tissue content

EXPLANATION OF FIGURES 5 TO 10

Fig. 5.—Section of liver with 3.5 per cent fibrous tissue. Hematoxylin and eosin; × 50.

Fig. 6.—Section of liver with 5 per cent fibrous tissue. Hematoxylin and eosin; × 50.

Fig. 7.—Section of liver with 6.3 per cent fibrous tissue. Hematoxylin and eosin; × 50.

Fig. 8.—Section of liver with 13.3 per cent fibrous tissue. Hematoxylin and eosin; × 50.

Fig. 9.—Section of liver with 17.5 per cent fibrous tissue. Hematoxylin and eosin; × 50.

Fig. 10.—Section of liver with 23 per cent fibrous tissue. Hematoxylin and eosin; × 50.

averaged 1.9 per cent of the dry liver weight, the range being from 0.8 to 2.8 per cent.

Figure 4 shows the histologic appearance of liver with 2.8 per cent · of fibrous tissue. This is essentially normal liver.
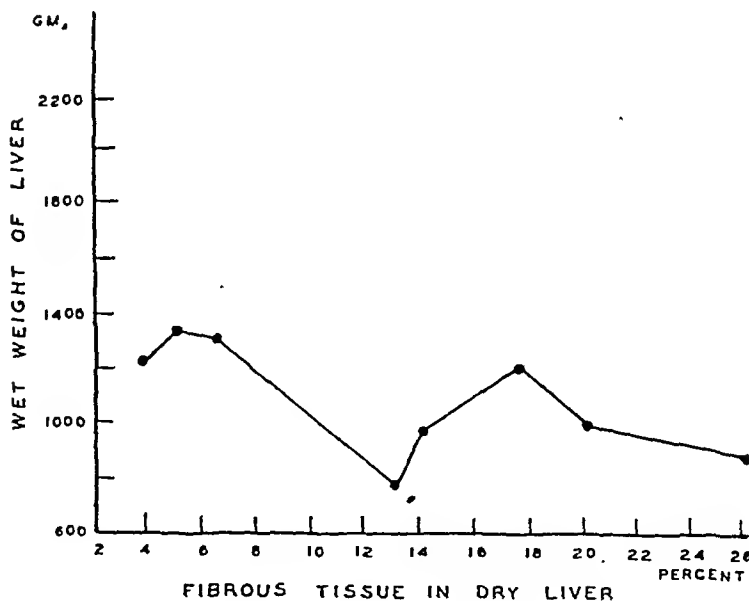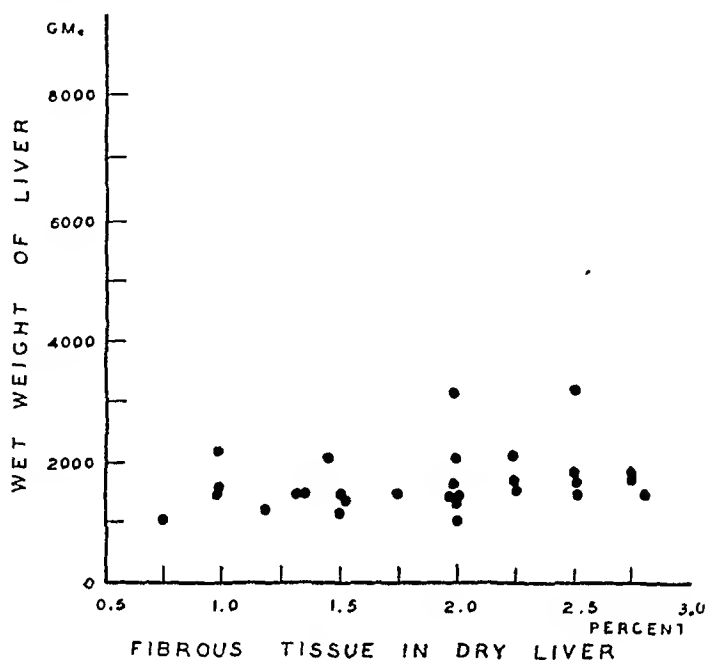
Fig. 11.—Weights of noncirrhotic livers with the corresponding percentages of their fibrous tissue contents.

Fig. 12.—Weights of fibrotic livers with the corresponding percentages of their fibrous tissue contents.

Weight of Liver and Amount of Fibrous Tissue.—Figures 11, 12 and 13 indicate the weights of noncirrhotic and cirrhotic livers and the corresponding percentages of fibrous tissue.

Figure 11 shows the weights and the percentages of fibrous tissue of noncirrhotic livers. It is clear that no relationship exists between the amount of fibrous tissue and the weight of the noncirrhotic liver. The outstanding examples were a liver which weighed 7,600 Gm. and had 1.5 per cent fibrous tissue, and another which weighed 1,010 Gm. and had 0.8 per cent fibrous tissue. The former was the liver of a person suffering from alcoholism and showed extensive fatty infiltration, which accounted for the increase of weight.[3]
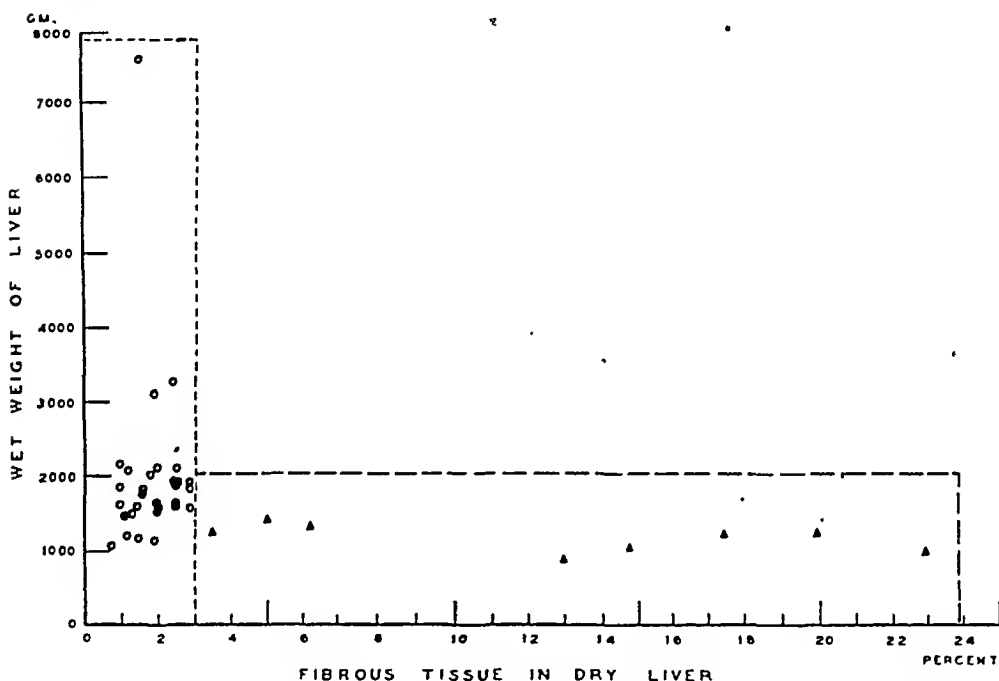
Fig. 13.—Weights of both the fibrotic and the noncirrhotic livers plotted with relation to their fibrous tissue contents.

Figure 12 shows the weights and the percentages of fibrous tissue of fibrotic livers. It clearly shows that there is a definite inverse relationship between the amount of fibrous tissue and the weight of the liver. As cirrhosis develops, the amount of fibrous tissue increases and the weight of the liver comes down.

---

3. While the stroma was apparently normal on the basis of the histologic examination, if the total liver weight was reduced to the normal of 1,500 Gm., the amount of fibrous tissue would be about three times the normal. This liver may well be precirrhotic.

The fibrous tissue contents of normal and fibrotic livers have been measured quantitatively. In livers which gave no evidence of hepatic damage, the average content was 1.9 per cent of the dry weight of the liver, the range being 0.8 to 2.8 per cent. In livers showing gross or microscopic evidence of fibrosis, the fibrous tissue content was from 3.5 to 23 per cent of the dry weight.

Histologic examination of liver sections showed that in the lower range of values of fibrous tissue there is fairly good correlation between the amounts of fibrous tissue determined quantitatively and the amounts seen in microscopic examination. For example, a liver with 3.5 per cent of fibrous tissue may be recognized as showing increased fibrous tissue microscopically (fig. 5). From 5 to 10 per cent fibrous tissue is well correlated with microscopic appearance. Above this, correlation is difficult, a liver with 23 per cent fibrous tissue being indistinguishable from one with 13.3 per cent fibrous tissue. Hence, microscopic examination alone may fail to give a correct idea of the extent of cirrhosis.

Figure 13 shows the weights and percentages of fibrous tissue of both the noncirrhotic and the fibrotic livers. It demonstrates that noncirrhotic livers show a fibrous tissue content of under 3 per cent, regardless of weight. The weights of those livers in which fibrous tissue is increasing tend to decrease as fibrosis becomes more marked.

There is no constant relationship between the weight of the nonfibrotic liver and the amount of fibrous tissue. In the small series of cases of hepatic fibrosis the weight of the liver decreased as the fibrous tissue content increased. The amount of fatty infiltration present, in association with cirrhosis, especially with the alcoholic type, may be a confusing factor. This will always send the weight of the liver up, and the percentage of fibrous tissue will not be a true indication of the extent of cirrhosis.

SUMMARY

The fibrous tissue contents of noncirrhotic, fibrotic and cirrhotic human livers have been measured by chemical means.

Good correlation was found between the amount of fibrous tissue microscopically visible and the weight of the liver in all cases of fibrosis except those in which fibrosis was extreme. As the amount of fibrous tissue increases, the weight of the liver decreases.

There was no apparent correlation between the amount of fibrous tissue and the weight of the liver in noncirrhotic cases.

PREINVASIVE CARCINOMA OF THE CERVIX UTERI

Seven Cases in Which It Was Detected by Examination
·of Routine Endocervical Smears

EDGAR R. PUND, M.D.

H. E. NIEBURGS, M.D.

J. B. NETTLES, M.D.

AND

J. D. CALDWELL, M.D.

AUGUSTA, GA.

INCIPIENT cancer of the cervix uteri is usually noninvasive, and when it is in this phase adequate therapy should result in cure in 100 per cent of the cases. In a statistical study of cases of cancer of the cervix, Pund and Auerbach [1] found that the average age of patients with preinvasive carcinoma was 36.6 years, six years below the average age of patients with covert invasive carcinoma and twelve years below that of patients with overt carcinoma. The detection of cancer which is in its preinvasive phase therefore offers a challenge to the physician. In general, preinvasive carcinoma gives rise to ·no symptoms and cannot be detected by physical examination. It can easily be missed in biopsy because of its limited extent and its endocervical location. The endocervical smear stained according to the method of Papanicolaou [2] offers the best routine method of detecting neoplastic cells of the cervix in their preinvasive phase; however, the diagnosis must be confirmed in biopsy or endocervical curettage. Several observers [3] have reported a few cases in which preinvasive carcinoma of the cervix was detected by examination of a vaginal smear. A study of routine endocervical smears has enabled us to report 7 additional cases.

1. Pund, E. R., and Auerbach, S. H.: J. A. M. A. **131**:960, 1946.

2. Papanicolaou, G. N.: J. A. M. A. **131**:372, 1946.

3. Meigs, J. V.: J. A. M. A. **133**:75, 1947. Ayre, J. E.; Bauld, W. A. G., and Kearnes, P. J.: Am. J. Obst. & Gynec. **50**:102, 1945. Meigs, J. V., and others: Surg., Gynec. & Obst. **81**:337, 1945. Papanicolaou, G. N., and Traut, H. F.: Diagnosis of Uterine Cancer by the Vaginal Smear, New York, The Commonwealth Fund, 1943. Fremont-Smith, M.: Graham, R. M.. and Meigs, J. V.: New England J. Med. **237**:302, 1947.

MATERIAL AND METHOD

Smears from patients attending the various departments of the clinic of the University Hospital and from private patients of physicians of Augusta and surrounding towns were submitted to this department for examination.   The method used for this study is simple and requires little time and effort on the part of the physician.   An ordinary cotton applicator is inserted into the cervical canal, twirled a few times in one direction and then rolled on a slide.   In everted cervices the applicator should also be rubbed against the area of eversion.   In addition a smear is made from the vaginal pool for study of endometrial cells and for study of the cytologic changes which reflect hormonal activity.   For vaginal films a speculum is not required.   The applicator is inserted deeply into the vagina, twirled and rolled on a slide.

The slides are immediately immersed in a solution of equal parts of ether and 95 per cent alcohol and should remain in this solution for at least fifteen minutes
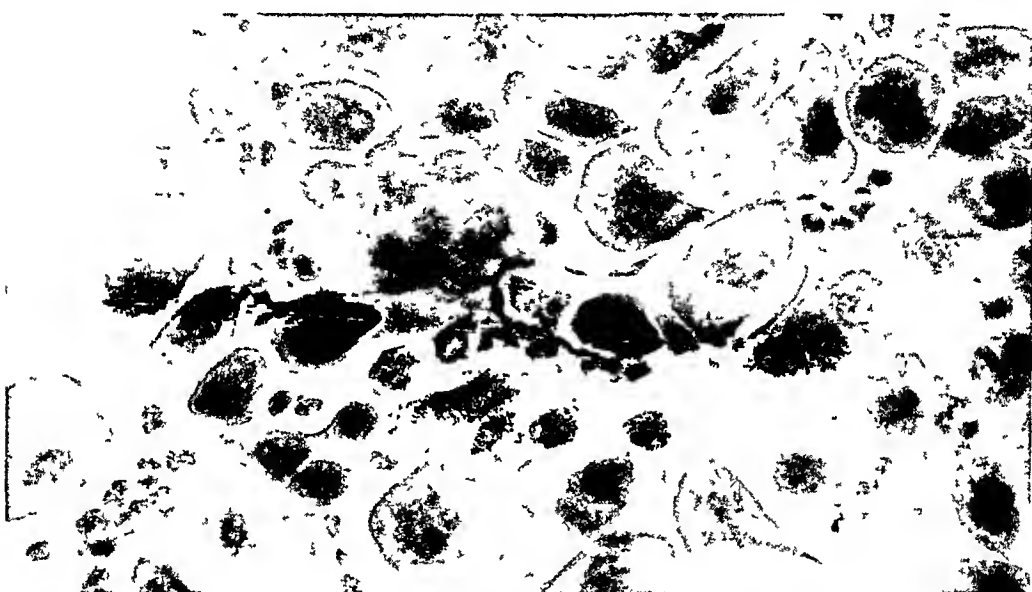
Fig. 1 (case 2).—Endocervical film.   The nuclei are hyperchromatic, disproportionately large and irregular in size and shape.   Compare the abnormal cells with the one normal cornified cell showing a small pyknotic nucleus.

but may remain there for several hours.   If the slides are to be sent to the laboratory, drying is prevented by the technic recommended by Ayre.[4]   One or two drops of glycerin should be placed on the film, and it is then covered with a clean slide, or two filmed slides are placed face to face.   The endocervical smears are stained by the method of Papanicolaou,[2] which affords the greatest transparency and the clearest nuclear detail.   Vaginal smears are stained for glycogen with hematoxylin and with Best's carmine.

REPORT OF CASES

CASE 1.—The patient was a white woman aged 72 years, a decipara.   Cervical biopsy and curettage of the uterus were performed at the time of perineorrhaphy.

---

4. Ayre, J. E : South. M. J. **39**:847, 1946

Clusters of cells suggestive of cancer were found in the stained endocervical films which had been secured before operation. In a biopsy specimen removed from the cervix preinvasive carcinoma was observed in one of the glands. This patient is being treated with radiation.

CASE 2.—A Negro woman aged 44, a unipara, complained of slight vaginal bleeding of six weeks' duration. Definite cancer cells were observed in vaginal and cervical films (fig. 1). A cervical specimen was obtained for biopsy, and the endocervix was curetted. The microscopic diagnosis was: "Chronic inflammation of the junctional endocervix of the small section of the cervix. The scrapings are from the endocervix, and among the fragments of normal squamous and glandular epithelium there are three shreds of squamous carcinoma which are noninvasive" (fig. 2). Three weeks later total hysterectomy was performed. Although in serial blocks taken from the junctional endocervix no definite cancer was found, regenerating squamous epithelium, in which there were foci of anaplasia, had begun to repair the denuded surface.

Fig. 2 (case 2).—Two shreds of cancerous squamous epithelium curetted from the endocervical canal. Note the irregularity in size of nuclei and the lack of differentiation of cells. The shreds of epithelium are limited by a basement membrane. Fragments of normal columnar epithelium are included in the curettings.

CASE 3.—A white woman aged 38, a quadripara, was referred to the cancer clinic because of irregular and excessive uterine bleeding. Few cells suggestive of cancer were found in the cervical film (fig. 3 A), but they were definitely abnormal. Preinvasive carcinoma was observed in three of the four sections of cervix (fig. 3 B) ; no carcinoma was seen in the endocervical curettings. This patient received 2,000 milligram hours of radium therapy, and hysterectomy was subsequently performed. No remains of cancer were found in serial blocks of the junctional endocervix.

CASE 4.—The patient was a white woman aged 32 years, a tripara. Abnormal cells were found in the endocervical film which was made by a private physician for routine study. Preinvasive carcinoma was found in one of three small sections

of cervix. No carcinoma was found in the endocervical curettings. At the time of the biopsy the cervix was extensively cauterized. After subsequent hysterectomy, no remains of the carcinoma were found in serial blocks of the cervix.

CASE 5.—A Negro woman aged 35, a tripara, complained of bleeding between periods for five months. On vaginal examination a superficial erosion was observed at the os of the slightly enlarged cervix. In two sections of the biopsy specimen of the cervix, preinvasive carcinoma was found, which involved the surface and the mouths of the glands. Over a period of a month the patient received 3,000 roentgens of high voltage roentgen radiation. Six· weeks later, on vaginal examination, the cervix appeared normal. In the endocervical smear, however, cancer cells were observed as well as minor radiation changes. Total hysterectomy
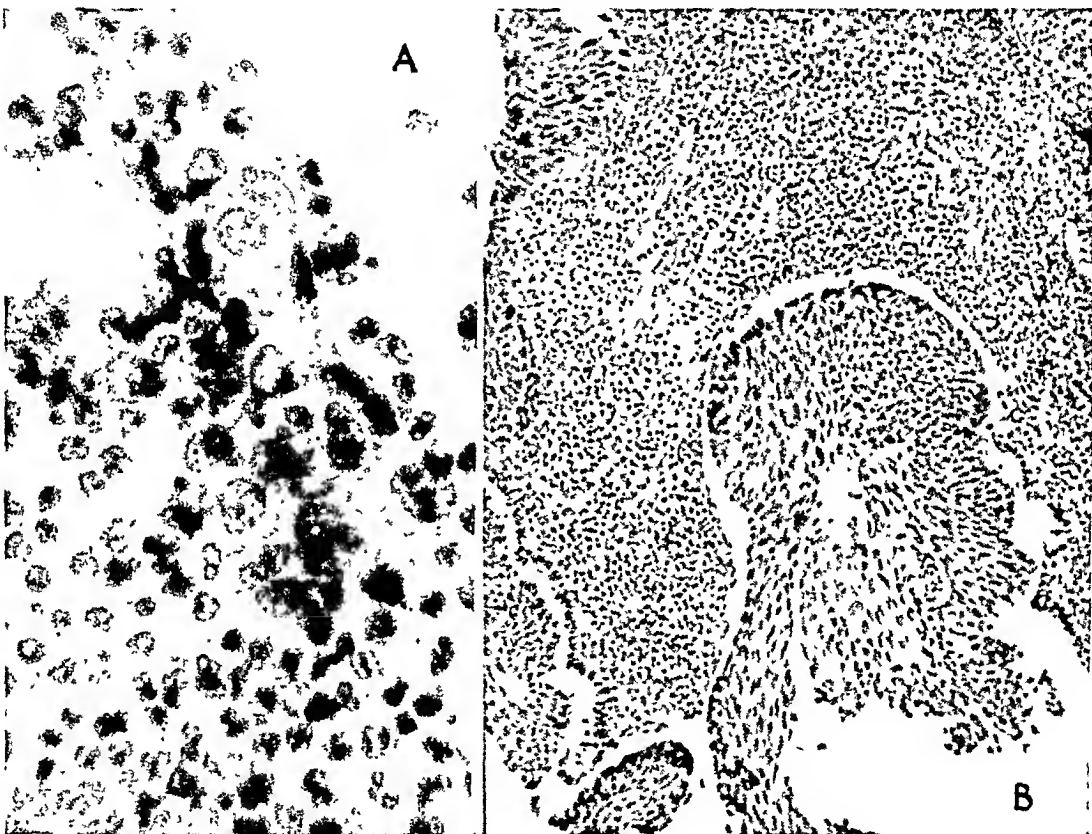
Fig. 3 (case 3).—*A*, endocervical film. Only a few atypical cells were observed. The nuclei are disproportionately large, vesicular, hyperchromatic and of varying size and shape. *B*, biopsy specimen. Undifferentiated squamous epithelium is limited to the surface and extends into the mouth of one gland.

was performed. A minute preinvasive carcinoma was found at the junctional endocervix in only one of several serial blocks.

CASE 6.—A white woman aged 33, a tripara, complained of leukorrhea. In the routine endocervical film definite cancer cells were found. Biopsy and endocervical curettage were performed. Preinvasive carcinoma was found in two of the three small sections of cervix, and slivers of cancerous epithelium were observed in the endocervical curettings. No invasion was seen. Total hysterectomy

was performed five weeks later, and carcinoma was present in two of ten blocks of junctional endocervix (fig. 4).

CASE 7.—A white woman aged 62 years, a secundipara, had noted "spotting" on one occasion. On vaginal examination, the cervix was seen to be everted, and the anterior lip at the site of eversion appeared thickened and vélvety. A small polyp projected from this lip, and an additional small polyp was present on the posterior lip. In the endocervical film, which in this case was prepared by scraping the endocervix with a wooden applicator, there were numerous shreds of atypical squamous epithelium. The nuclei were hyperchromatic, disproportion-
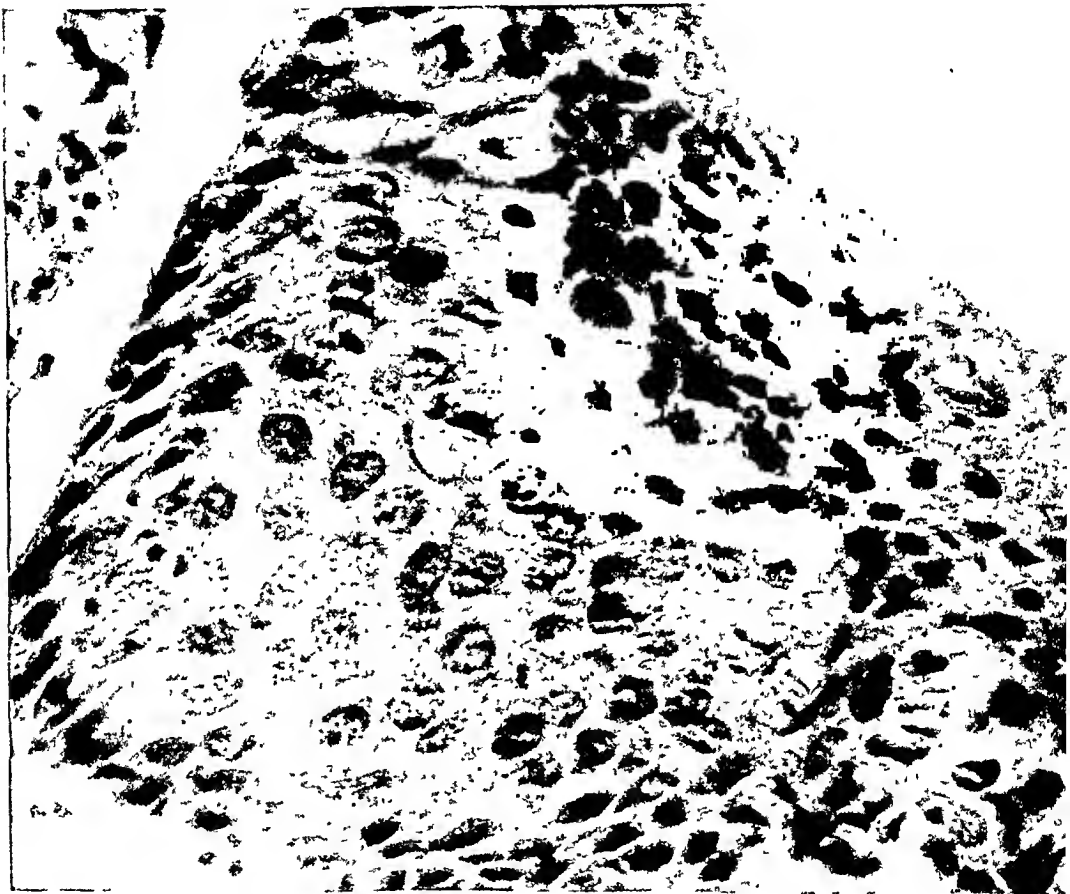
Fig. 4 (case 6).—High magnification of preinvasive carcinoma found in a section of cervix after total hysterectomy. The presence of this carcinoma was suggested by an endocervical film, and the neoplasm was demonstrated in a biopsy specimen. The cells are distinctly neoplastic, although there was no invasion. The nuclei are vesicular, hyperchromatic, disproportionately large and irregular in size. Cellular differentiation is incomplete.

ately large and variable in size, and numerous mitoses were seen. There was no evidence of polarity. Free atypical cells were also observed. The cells were so characteristic of cancer that biopsy was recommended, and radium therapy was instituted at the time of biopsy. A generous biopsy specimen of the anterior lip included the attached polyp, and the other polyp was removed. No curettings could be obtained from the body of the uterus. In two of eight microscopic sections of the biopsy specimen small foci of preinvasive carcinoma were observed. Much of the surfaces had been denuded by the applicator and by surgical preparation.

COMMENT

Our observations confirm those of others that preinvasive carcinoma can be detected by the examination of vaginal and endocervical spreads. The presence of atypical cells, however, is not always indicative of cancer. We wish, therefore, to emphasize that it is necessary to confirm the diagnosis by histologic sections. Biopsy of two or more sites should be carried out and care exercised to choose the proper sites—the endocervical junction at the external os. Due allowance must be made for variations when ectropion is present. We have recently emphasized the importance of endocervical curettage.[5] Cancers may be detected in this manner when not found in biopsies (case 2, fig. 2). Conversely, however, the endocervical scrapings may be negative in the presence of a positive cervical biopsy specimen (cases 3 and 4). It is therefore recommended that biopsy specimens and endocervical curettings be obtained. Since we are recommending total hysterectomy without ovariectomy for the younger age group of patients with preinvasive carcinoma, the endocervical curettage also serves to differentiate the preinvasive carcinoma from covert invasive carcinoma.

In our study we prefer at the present time the swab method of obtaining the smear from the endocervix. The junctional endocervix is generally the site of the incipient cancer, and we assume that more cancer cells would be found there than in the vaginal smears. However, vaginal smears should also be made at the same time for the study of the cytologic changes which are caused by the hormones and also for the detection of abnormal endometrial cells. With the swab method, however, cancer cells are not found in large numbers, and careful prolonged examination is necessary. Normal cells constitute the chief cells of the film, and only a few small foci of cancer cells may be observed. For our purpose we preferred the cotton swab rather than a scraping instrument. We initiated this study for the detection of preinvasive cancers, and we desired confirmation with histologic preparations. We therefore feared the risk of denuding the cervix of its atypical epithelium prior to biopsy. By preserving the surface epithelium we have also been able to explain from subsequent biopsies and curettage some of the "suspicious" films. The chief offenders of this group we found to be healing ulcerations, imperfect metaplasia in estrogenic deficiency and metaplastic epithelium with superficial maceration.

It has been demonstrated that some preinvasive carcinomas can be detected by vaginal and endocervical smears. Further study however

5. Pund, E. R., and others: Preinvasive and Invasive Carcinoma of Cervix Uteri: Pathogenesis, Detection, Differential Diagnosis and the Pathologic Basis for Management, Am. J. Obst. & Gynec., to be published.

is necessary to determine whether all cancers of the cervix can be diagnosed in their incipiency. When we have accumulated sufficient material, we shall attempt to analyze our results and compare the incidence with that of Pund and Auerbach,[1] who reported that preinvasive carcinoma occurred in 3.9 per cent of 1,200 surgically removed cervices. Because the incipient cancers are frequently asymptomatic and grossly invisible, the making of vaginal and endocervical smears should be a routine procedure in physical examinations, especially in those of parous women. In this way a larger number of cervical carcinomas will be diagnosed in their curative stage.

SUMMARY

Seven cases in which preinvasive carcinoma of the cervix was detected by study of endocervical smears are reported.

The importance of making routine endocervical smears for the detection of incipient cancer of the cervix is discussed.

In cases in which the smears show cancer cells or cells arousing suspicion of a cancerous change, the diagnosis of cancer of the cervix should be confirmed by biopsy.

The value of endocervical curettage is emphasized.

RARE ANOMALY OF THE VERMIFORM APPENDIX

Mucous Lining of the External Surface

PROFESSOR DR. ST. MAHRBURG
LUBLIN, POLAND

DISTURBANCES of development consisting in improper lodgments of elements of mucous membrane usually concern the female generative organs. There is displacement of larger or smaller clusters of endometrium—endometriosis; seldom seen is the group of endometrial cells displaced to the peritoneal uterine·covering, called endometriosis externa, or the cul-de-sac, occasionally encountered on the broad or the round ligament.

Rarely observed are transplantations of elements of other mucous membranes, as elements of the intestinal tract misplaced in the outer wall lining—enterocystoma—and usually considered as remnants of the fetal circulation—ductus omphalomesentericus—or of the pancreatic tissue. Spreading in the intestinal wall, the glandular elements may lodge under the peritoneal cover or in the muscular layer of the intestine, causing glandular enlargements—adenomyomatosis. Such changes may simulate a new growth, giving cause for surgical intervention.

Rare are the observations pointing to elements of the intestinal inner lining displaced to the peritoneal surface: The formation of a membrane on the surface of a peritoneal coat may have an inflammatory origin. The place at which such changes most frequently occur on the intestinal tract is the appendix.

The reporting of the case observed by me is worth while not only because a membrane, tunica mucosa, was abnormally placed and formed on the outer surface of the appendix but because it covered the entire appendix, giving an impression of. a normal appearance and suggesting numerous questions as to the nature of its development and the associated pathologic condition. In the literature that was available to me. I could not find any description of such a displacement of mucous membrane.

A man aged 28, a policeman, was admitted to St. Jacob's Hospital, Vilna, Poland, May 2, 1942. He was well built and well nourished. He complained of pain in the abdomen and slight nausea. The history and the physical findings were essentially irrelative. His temperature was 99.6 F. A diagnosis of subacute

appendicitis was made, and after induction of local anesthesia, the appendix was removed. There were no complications during the operation, and no adhesions were found. The postoperative course was uneventful. The incision healed by first intention, and the patient left the hospital on the tenth day, in good condition. So far the clinical picture is that of the common subacute appendicitis. The removed appendix was sent to the histopathologic department.

Macroscopically, the specimen was of normal thickness and about 10 cm. long. The surface appeared somewhat velvety and rather pale. The tip was slightly inflamed and the attachment of the mesoappendix well defined.

Microscopically, under low power, the cross section readily revealed an abnormal construction, namely, the placement of mucous membrane, instead of the serous coat, as the outer lining of the appendix. It was really a second mucosa, differing in no way from that lining the inside of the appendix. The external mucosa covered all of the appendix except the mesoappendix. Also with low power it was plainly seen that the glands of the outer lining penetrated the wall of the appendix, forming a junction between the inner and outer linings, and the glands
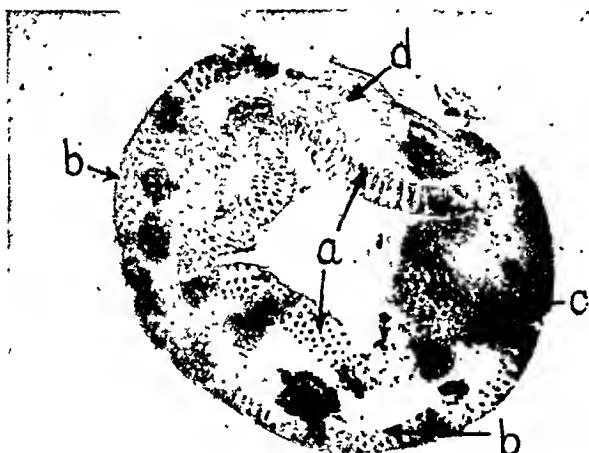
Cross section of the appendix; × 60: (*a*) Normal mucosa. (*b*) Mucosa as outer coat. (*c*) Juncture of both mucous membranes. (*d*) Place at which meso-appendix was attached.

of both the inner and the outer mucosa were of the same type. The covering layer of these glands consisted of cylindric cells. These cells were swollen and evenly distributed over the basic membrane. A large number of cuboidal cells, filled with cytoplasm, gave the picture of active mucosal cells. The nuclei of the cells were well visualized and normally placed. There were a few erythrocytes attached to the cell membranes. Between glands the texture was edematous; in the outer layers were minute hemorrhagic spots. The arterioles were dilated, and numerous absorbing cells were seen. In the wall of the mucosa, between the glands, many granules were noted; some were close to the surface of the mucosa, while others, in groups, were penetrating into the deeper layers.

The submucosa was thin: The muscle cells were well defined, but the inner layer was not so well developed. The submucosa of the normally placed mucous membrane was wide, containing many dilated blood vessels, also absorption spaces. The basement membrane was of a hemorrhagic type. Numerous sections of the glands contained many cuboidal cells in active condition. The stroma or tunica

propria contained many squamous cells. In the lumen of the appendix, and also on the outer covering, were seen clusters of erythrocytes.

Changes in both mucous membranes (the dilated blood vessels and the free erythrocytes) speak for subacute inflammation. These changes were alike in both mucosae, the inner and the outer.

The anomalous mucous membrane must have formed over the appendix during embryonic life, before the formation of the primary intestinal tract. It is important to remember that during the evolutionary stages the elements of mucosa lodged in the place of the serosa continued to grow normally in the early development of the fetal life, giving the picture of normal mucosa. That mucosa, placed over the outer surface of the appendix, developed fully, regardless of the serosa of adjacent structures and the entirely different function of its natural habitat, and retained the formation and consistency of intestinal mucosa for a period of twenty-eight years. In spite of the inflammatory condition observed, the surrounding tissues did not react adversely, as no adhesions were found during operation.

Although this report does not present an exhaustive picture, as it concerns only the biopsy aspects of the question, and nothing is known about the condition of the serous membranes adjacent to the appendix, it does point to the possibility of development and existence of a considerable extent of mucosa of the digestive tract in an atypical place.

PRIMARY ATYPICAL PNEUMONIA

Report of Eight Cases with Autopsies

FREDERIC PARKER Jr., M.D.

LESLIE S. JOLLIFFE, M.D.
AND
MAXWELL FINLAND, M.D.
BOSTON

ALTHOUGH cases of primary nonbacterial pneumonia of unknown but probably viral cause have been recognized in great numbers during recent years,[1] in most of them the disease has been mild or of only moderate severity. Fatal cases have not been frequent, and only a few descriptions of the pathologic changes in such cases are available.[2] A group of 8 such cases in which autopsies were done is therefore of interest. In all these cases there was a characteristic clinical course with similar physical, roentgenologic and pathologic findings in the lungs and, in addition, there were cold agglutinins in the serum. All but one of them occurred in the latter months of 1942.

REPORT OF CASES

CASE 1.—This case is reported in more detail elsewhere because the pneumonia was associated with a bullous type of erythema multiforme.[3] The illness in this 17 year old boy began on Aug. 12, 1942, with coryza and malaise, followed in a week by a cough productive of gray sputum. On August 25 he had a chill and began to have a sore throat, dysphagia, blood-streaked sputum and pain in the anterior region of the chest. The course of the illness was characterized by an extensive bullous eruption of the skin and of the mucous membranes of the orificial surfaces, severe conjunctivitis and the symptoms, signs and roentgenologic findings

1. Reimann, H. A.: Medicine 26:167, 1947. Dingle, H. J., and Finland, M.: New England J. Med. 227:378, 1942.

2. (a) Longcope, W. T.: Bull. Johns Hopkins Hosp. 67:268, 1940. (b) Kneeland, Y., and Smetana, H. F.: ibid. 67:229, 1940. (c) Campbell, T. A.; Strong, P. S.; Grier, G. S., III, and Lutz, R. J.: J.A.M.A. 122:723, 1943. (d) Golden, A.: Arch. Path. 38:187, 1944. (e) Meiklejohn, G.; Eaton, M. D., and van Herick, W.: J. Clin. Investigation 24:241, 1945. (f) Stanyon, J. H., and Warner, W. P.: Canad. M. A. J. 53:427, 1945.

3. Finland, M.; Jolliffe, L. S., and Parker, F., Jr.: Am. J. Med., to be published.

of diffuse atypical pneumonia. Oxygen and sulfadiazine and tube feedings with vitamin supplements were of no avail. The patient became increasingly dyspneic, cyanotic and delirious and died on September 3. The only significant laboratory findings were: white blood cell counts ranging mostly between 12,200 and 17,500, with 85 to 60 per cent polymorphonuclear neutrophils; sputum cultures which showed alpha hemolytic streptococci in varying numbers and Staphylococcus aureus in increasing numbers; a psittacosis complement fixation titer of 1:256 (4 plus) for the serum on August 28 and September 3 and a cold agglutinin titer of 1:640 on the latter date.

Autopsy (eighteen hours after death).—Over the entire body there were lesions varying from 0.5 to 2 cm. in diameter; some were blebs which contained clear fluid, but many were dried, and others, including those on the lids, the scrotum and the penis, appeared ulcerated and covered with hemorrhagic crusts. There was congestion of the conjunctival vessels, with some conjunctival hemorrhages.

The gross appearance of the lungs is shown in figure 1. The combined weight of the lungs was 1,200 Gm. There was a thin layer of fibrin over the lower lobe of the right lung and over both lobes of the left lung. The middle lobe was subcrepitant and had a white surface, but all the other lobes of the lungs showed marked decrease to absence of crepitation and were purple-red. The cut surfaces were dark red-purple, and no purulent material could be expressed; they presented an appearance of miliary nodules and showed some dark areas of congestion and atelectasis, especially in the posterior and dependent portions. The mucosa of the trachea and bronchi was congested. The tracheobronchial lymph nodes were not enlarged. There was some congestion of the cerebral veins. The remaining organs appeared grossly normal.

Microscopic Examination.—The observed changes varied greatly in different areas of the lungs. In some areas the alveoli were essentially normal; in others they contained an albuminous precipitate, and in still others there were fibrin and red blood cells. The lining cells of many alveoli were swollen and vacuolated, and a few showed mitotic figures. In such areas the exudate in the alveoli consisted of desquamated alveolar lining cells, plasma cells, other mononuclear cells, an occasional multinucleated cell and a rare giant cell of the foreign body type (fig. 2 B). In some places the bronchi contained numerous polymorphonuclear leukocytes and some clumps of cocci (fig. 2 A). The peribronchial and perivascular regions were infiltrated by plasma cells, some lymphocytes, rare mast cells and eosinophils, and some large immature cells of an unidentified type. In the submucosa of the trachea and about the glands there was an infiltration in which lymphocytes, many plasma cells and an occasional mast cell took part.

A section of the skin showed a vesicle with necrosis of the covering epithelium and a base consisting in part of intact epithelium and in part of necrotic connective tissue infiltrated by polymorphonuclear leukocytes and large mononuclear cells. The vesicle contained precipitated albumin and fibrin in one part and polymorphonuclear leukocytes, large mononuclear cells, fibrin and a moderate number of cocci in another. Some of the hair follicles and coil glands were necrotic. In the deeper portions of the corium there was a perivascular infiltration of large mononuclears, lymphocytes, plasma cells and an occasional eosinophil and mast cell. The blood vessels in the necrotic connective tissue were thrombosed.

There were a few scattered large mononuclear cells, lymphocytes and plasma cells in the cerebral meninges, and similar cells infiltrated the interstitial tissues of other organs.

Bacteriologic Study.—Cultures of the heart's blood showed no growth. Hemolytic Staph. aureus was cultured from the lower lobe of the right lung, the lower
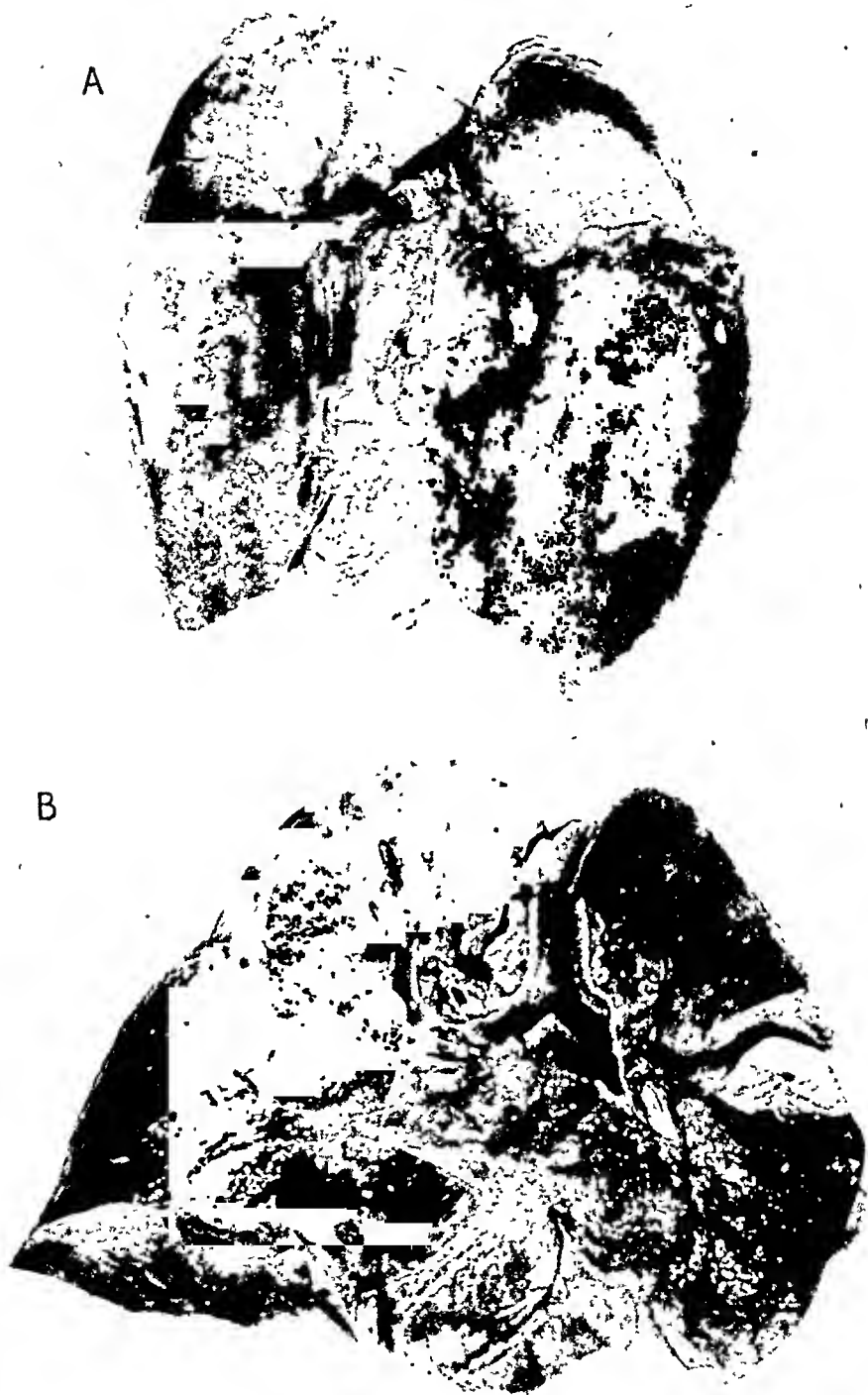
Fig. 1 (case 1).—*A*, posterior view of the lungs, showing the sharply demarcated dark areas of atelectasis of the lower lobes and the pale gray surfaces of the emphysematous upper lobes. (The shiny white areas are high lights.) *B*, cross section of these lungs. The cut surfaces show numerous small gray nodules, surrounded by darker congested areas, in the lung parenchyma. The mucosa of the trachea and bronchi is deeply injected. (The white areas are high lights.)
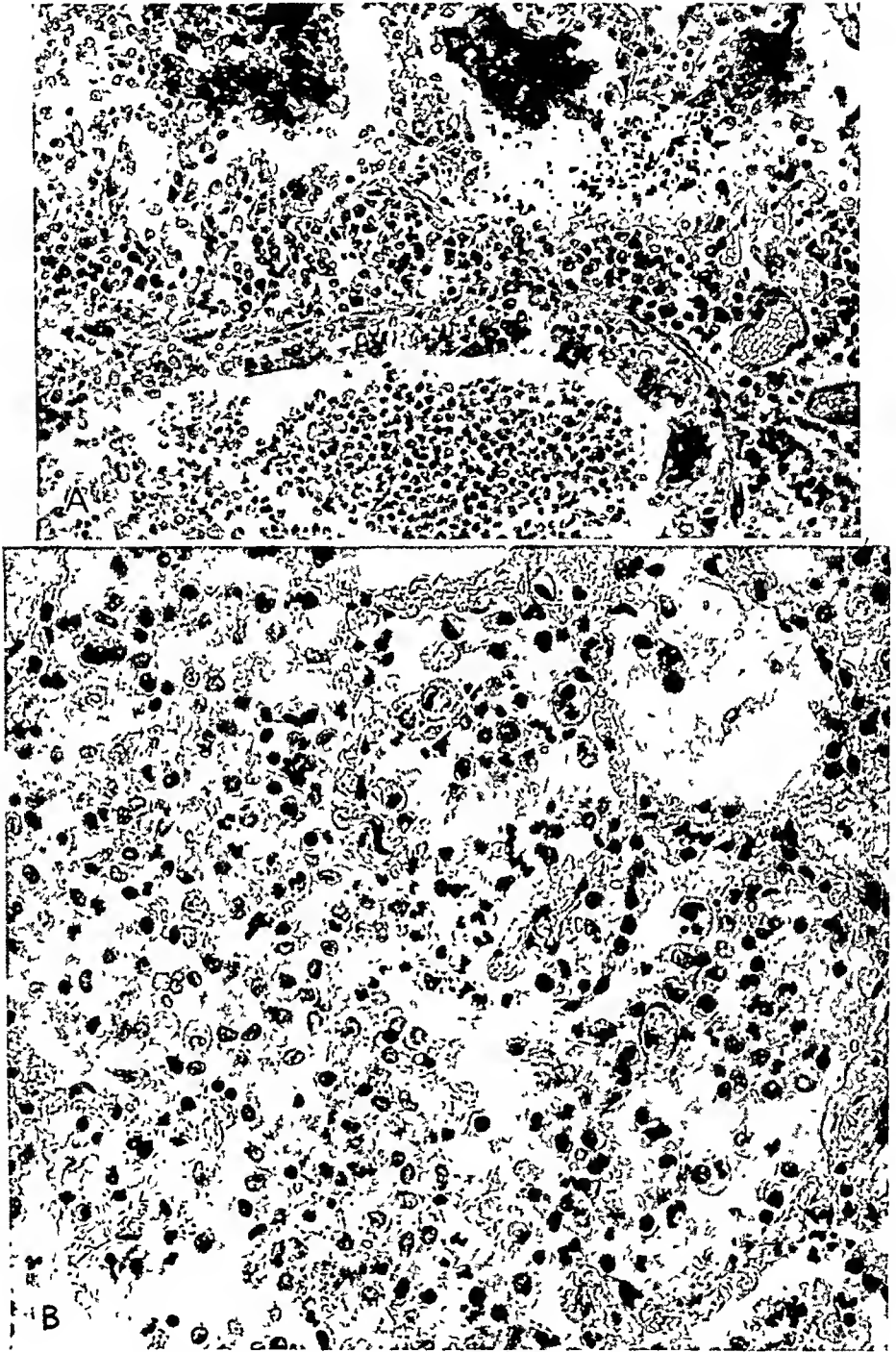
Fig. 2 (case 1).—*A*, bronchiole containing polymorphonuclear leukocytes in its lumen. Its wall is infiltrated with mononuclear cells. The adjacent alveoli contain fibrin and large mononuclear cells. Phloxine–methylene blue stain; × 100.

B, alveolar lumens containing an exudate of mononuclear cells. Phloxine–methylene blue stain; × 400.

lobe of the left lung, the pleura and the liver, and the last contained an enterococcus in addition. Mice and embryonated eggs inoculated with vesicle fluid and suspensions of the lungs have yielded no virus that could be identified.

CASE 2 (courtesy of Drs. Henry D. Stebbins and D. A. Nickerson).—A 50 year old white married woman began to have chilly sensations and general malaise on Sept. 27, 1942. On the following day she had a nonproductive cough, and on each of the next two days she had shaking chills, each followed by a rise of temperature to 103 F., but her physician found no abnormal signs in the chest. He sent her to the Salem Hospital on September 30. There was a vague history of mild asthmatic attacks in the past. A few years previously she had some urinary symptoms, but studies at that time revealed normal renal function.

At the hospital physical examination gave essentially negative results except for the fever. The hemoglobin content was 86 per cent; the red blood cell count, 4,160,000; the white cell count, 4,900, with 68 per cent polymorphonuclear neutrophils. The initial examination of the urine showed albumin (3 plus), with a specific gravity of 1.013 and a normal sediment. The blood nonprotein nitrogen was 42 mg. per hundred cubic centimeters. A blood culture was negative. Roentgenograms of the chest showed diminished radiance in the right lung field with linear, mottled and patchy densities in the lower half of this lung and a few small nodular areas on the left.

The patient was given sulfathiazole, 2 Gm., followed by 1 Gm. every four hours, together with an equal amount of sodium bicarbonate. This was discontinued on October 2 because of increasing vomiting, restlessness and headache and finally the appearance of a maculopapular rash. On October 10 the patient was given sulfadiazine, 1 Gm. every four hours, and on October 12, because of low blood concentrations, she received an intravenous injection of 5 Gm. of sodium sulfadiazine in a liter of 5 per cent dextrose. This was repeated twelve hours later. No further sulfonamide therapy was given. The fluid intake, part of which was given parenterally, ranged from 2,500 to 5,000 cc. per day, and the urinary output corresponded. The patient was kept in an oxygen tent continuously after October 2 except for a few occasions when pure oxygen was given by mask in attempts to relieve severe dyspnea. Aminophylline, in doses of 0.5 Gm., and epinephrine, 0.2 to 0.5 cc. of a 1:1,000 solution, were given for dyspnea that was associated with wheezing. Codeine, barbiturates and occasional small doses of morphine were given as sedatives. On October 11 a plasma transfusion was followed by a severe chill. After October 12 she received daily transfusions of 250 to 500 cc. to a total of 2,500 cc.

The patient's course was a stormy one. For the first ten days in the hospital, the fever was irregularly sustained, with peaks of 102 to 104 F., the pulse rate ranged between 100 and 120 and the respiratory rate between 25 and 30 per minute. There were three severe chills during this period. On October 11 the temperature dropped and stayed below 101 F., but the pulse rate and respiratory rates rose. The blood pressure was 140 systolic and 80 diastolic and gradually dropped to 110 systolic and 60 diastolic. Dyspnea and cyanosis increased steadily, with periods of asthmatic wheezing and severe labored breathing, which were only partly relieved by epinephrine and aminophylline. Rales appeared in the lungs soon after entry and increased until both lungs were filled with medium and coarse crepitant rales, and there were scattered musical rales during the periods of air hunger. No definite areas of consolidation were made out, however. The patient became increasingly stuporous and was unconscious during most of the latter half of her stay in the hospital. She died on October 18.

The white blood cell count was 4,700 on October 2, but the polymorphonuclears rose to 85 per cent. On October 5 the leukocyte count rose to 7,900, then to 24,100 on October 9, and thereafter ranged from 25,000 to 32,000, with 92 to 96 per cent polymorphonuclears, most of which were young forms and contained toxic granules. On October 9 the scleras were noted to be icteric, and the icterus index on that day was 14. On October 12 the hemoglobin content was 44 per cent, and on the following day it was 37 per cent, with a red blood cell count of 1,520,000. After transfusions the hemoglobin rose steadily to 84 per cent and the red blood cell count to 3,780,000. The urine, examined at four day intervals, showed a specific gravity of 1.009 to 1.013 and albumin (2 to 3 plus), with a few white blood cells and rare red blood cells in the sediment. On October 8 a test of the urine for bile was recorded as positive, and there was an unusually large amount of albumin in the same specimen. The blood nonprotein nitrogen stayed between 32 and 44 mg. per 100 cc. until October 10; the following day it rose to 75 mg. and thereafter fluctuated between 80 and 130 mg. The blood sulfathiazole level on October 2, just before that drug was stopped, was 6 mg. per 100 cc. Sulfadiazine levels on October 10 and 12 were 5 and 13 mg. per 100 cc., respectively. Serum proteins ranged from 5.1 to 5.9 Gm. per 100 cc. The stools were negative for occult blood. On October 10 a sputum culture showed numerous hemolytic staphylococci (Staph. aureus). Blood cultures made on October 5 and 10 were negative. There was difficulty in grouping the blood prior to transfusion, and it proved to be due to cold agglutinins, which were present in a titer of 1 : 160 on October 9 and 1 : 1,280 on October 16. When tested in the warm state the blood was found to be group O. Because of the marked leukocytosis and the appearance of icterus on October 8 and the later discovery of severe anemia, it was assumed that acute hemolytic anemia developed on Octboer 7 or possibly one or two days earlier. Hemoglobinuria was not made out, but the urine of October 8 gave a stronger than usual reaction for albumin. Successive roentgenograms of the chest showed increasing amounts of miliary mottling of both lung fields with some larger irregular areas of density, which varied in location in the different films (fig. 3).

Autopsy (one and one-half hours after death).—Each pleural cavity contained approximately 100 cc. of turbid amber fluid. There were no adhesions. The left lung weighed 600 Gm. and the right 875 Gm. The pleural surfaces of the upper lobes were pale gray to reddish gray. The lower lobes and the dependent portion of the middle lobe of the right lung showed extensive areas of dark bluish purple parenchyma, with only small portions of paler gray and reddish gray tissue in the anterior portions. Showing throughout the pleural surfaces, particularly those of the upper lobes, were numerous small reddish black areas, averaging 2 to 3 mm. in diameter, in many of which there was a small pale yellowish red central nodule. These areas were present throughout the lung and on palpation produced a fine granular feeling. On section the cut surfaces were moist, reddish to crimson and studded with numerous dark, reddish black, somewhat irregular areas, varying from 2 to 5 mm. in diameter, which in a few instances had yellowish nodules similar to those seen in the pleura. Rather large quantities of frothy serosanguineous fluid could be expressed from the surfaces. The bronchi and trachea were lined with dusky, yellowish tan mucosa covered with moderate amounts of thin mucoid material.

Microscopic Examination.—In sections of the lungs many alveoli contained numerous desquamated alveolar lining cells, some of which had vacuolated cytoplasm and the majority of which contained carbon. Some of these cells were binucleate and an occasional one was multinucleate. Practically all the alveoli

containing these cells also contained other mononuclear cells and red blood cells (fig. 4). Other alveoli contained precipitated albumin and desquamated alveolar lining cells. A few alveoli were filled with polymorphonuclear leukocytes, and here numerous cocci were present. An occasional alveolus showed organization. Some bronchioles contained polymorphonuclear leukocytes and cocci, and several showed beginning organization of the intraluminar exudate. There was a peribronchial and perivascular infiltration of plasma cells, some lymphocytes and in places polymorphonuclear cells. Several small arteries contained partially organized thrombi.

The spleen had numerous plasma cells and polymorphonuclear leukocytes in its pulp In addition, there were myelocytes and occasional focal collections of erythroblasts. The liver showed a rare small area of focal necrosis infiltrated by lymphocytes, plasma cells and polymorphonuclears. In the kidneys some glomeruli contained an increased number of polymorphonuclear cells in the capillaries in foci. Many tubules contained red blood cells and sometimes also a few polymorphonuclear leukocytes. The interstitial tissue was infiltrated in places by plasma cells, some lymphocytes and an occasional large mononuclear cell.
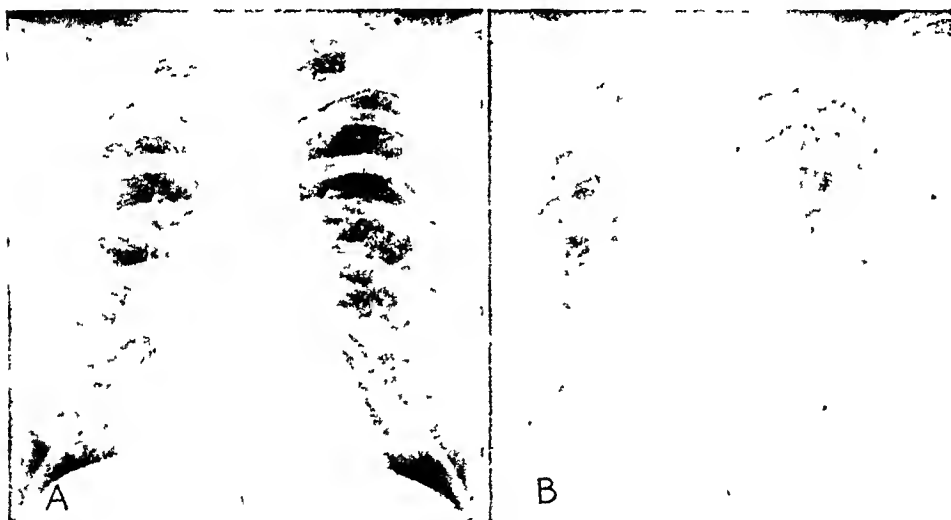
Fig. 3 (case 2).—*A*, roentgenogram of the chest taken on admission. It shows finely mottled densities extending from the right hilus.

B, roentgenogram of the chest taken two weeks after admission, showing miliary soft areas of density throughout both lung fields.

Bacteriologic Study.—In cultures all lobes yielded hemolytic Staph. aureus, occasional colonies of alpha hemolytic streptococci and rare colonies of Hemophilus influenzae. Mice and embryonated eggs inoculated with filtered suspensions of the lungs yielded no recognizable virus

CASE 3 (courtesy of Drs. Henry D. Stebbins, Wayne Hobbs and D. A. Nickerson).—A 53 year old white single woman was well until Oct. 6, 1942, when she began to have symptoms of "grip," with cough, malaise, prostration and a temperature of 102 F. These symptoms continued until she was seen by a physician, on October 12, when she was found to have a temperature of 101 F. and a few rales in the lower lobe of the right lung. She was given sulfadiazine, which she took irregularly until she was sent to the Salem Hospital on October 15. At this time the patient was moderately dyspneic and cyanotic, and showers of rales were heard over the lower lobes of both lungs. The blood sulfadiazine level was

2.4 mg. per 100 cc.; the hemoglobin content, 57 per cent; the red blood cell count, 2,850,000; the white cell count, 28,300, with 77 per cent polymorphonuclears; the icterus index, 18, and the blood nonprotein nitrogen, 42 mg. per 100 cc.

At the hospital, sulfadiazine therapy was continued, 1 Gm. being given every four hours, and the blood level on the day following admission was 12 mg. per 100 cc. The patient was placed in an oxygen tent, but the dyspnea was not relieved. Rales increased until they were heard throughout both lungs. The patient's temperature was 100 F. (rectal) or lower except for a brief rise to 103; the pulse rate was 120 to 140 and the respirations were 40 to 50 per minute most of the time and 60 to 70 during the last day.

On October 16 the urine was noted as being red and as showing albumin (1 plus), a positive reaction for bile and a few white blood cells in the sediment. The blood nonprotein nitrogen stayed between 42 and 48 mg. per 100 cc. A blood culture was negative. Roentgenograms showed diffuse miliary mottling of both lungs throughout (fig. 5). On October 17, the hemoglobin content dropped to 41 per cent, and the white blood cell count rose to 41,500 and on the following
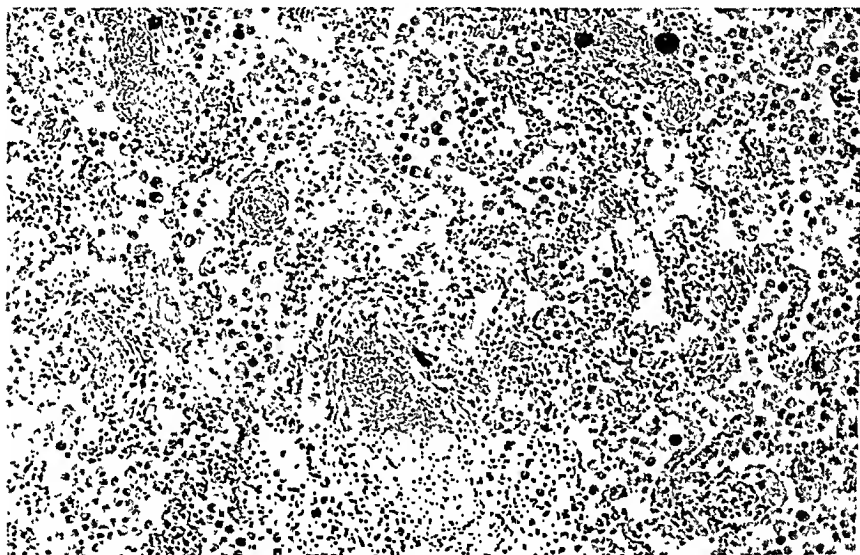
Fig. 4 (case 2).—Alveoli containing an exudate of mononuclear cells. Giemsa's stain; × 100.

day to 57,000, 92 per cent of which were polymorphonuclears. The red blood cell count dropped to 1,200,000 during this time. There was difficulty in typing the blood and in doing cross matchings for transfusions. It was assumed that the patient had a hemolytic type of anemia associated with cold agglutinins, but tests for cold agglutinins were not done. A transfusion was given on October 19. On that day the patient's dyspnea and cyanosis increased; she became markedly delirious and died shortly after the transfusion was completed.

Autopsy. (one and one-half hours after death).—The left lung weighed 725 Gm. and the right 660 Gm.; both were grayish and of a rubbery, firm consistence except at the apexes and the bases, which were fairly well aerated. The tissue cut with moderate resistance, revealing a gray surface, from which exuded large amounts of serosanguineous fluid. The cut surfaces were mottled with miliary punctate areas of consolidation spreading out from the hilar regions toward the periphery. The bronchi and trachea contained large amounts of serosanguineous fluid.

Microscopic Examination.—In sections of the lungs some alveoli contained precipitated albumin; others, desquamated alveolar lining cells, monocytes and fibrin, most of which was old and in places was undergoing organization. The lining cells of many alveoli were swollen, and an occasional one was in mitosis The alveolar walls were infiltrated by plasma cells, monocytes and a few polymorphonuclear leukocytes. Some bronchioles contained mucus and a few polymorphonuclear and mononuclear cells; others were empty. There was a perivascular and peribronchial infiltration of plasma cells. The septums were edematous and were infiltrated by plasma cells and large mononuclear cells. An occasional alveolar capillary was thrombosed.

There was an increased number of plasma cells in the pulp of the spleen. There was an occasional necrotic liver cell invaded by polymorphonuclear leukocytes. Some Kupffer cells contained polymorphonuclear leukocytes and red blood cells. The sinuses of the lymph node contained considerable numbers of mononuclear

Fig. 5 (case 3).—Roentgen appearance of the lungs on the day before death. There are soft nodular densities in both lungs.

cells, of which some had phagocytosed other cells and some contained carbon. There was an increased number of plasma cells in the lymph cords. The adjacent connective tissue was infiltrated by plasma cells and large mononuclear cells

Case 4.—A 52 year old white American housewife was in good health until Sept. 25, 1942, when she had aching in the back of her neck and a temperature of 101 F. On the following day she felt "all in" and took to bed. A physician found her temperature to be 103 F. and told her to rest in bed and take the antipyretic drugs which he prescribed For the next two weeks she complained only of weakness. On October 7 she tried to stay up but felt very dyspneic and had to return to bed During the next week moderate anorexia developed, and a cough productive of thick yellow sputum, which on two occasions was blood streaked She also had soreness in the left anterior region of the chest on coughing. She felt cold frequently and perspired at night but had no shaking chills. She was

admitted to the Boston City Hospital on October 14. The past history and the family history were noncontributory. Some of her neighbors had recently been ill with "flu," and her daughter was admitted at the same time with characteristic signs and symptoms of primary atypical pneumonia, which began October 11.

The patient was moderately obese, and on admission she was acutely ill and apprehensive. Her breathing was rapid, short and jerky. Her skin was warm and dry, and her lips and nail beds slightly cyanotic. The pharynx and fauces were red and injected but without exudate. There were moist rales scattered throughout both lungs; in the right lung, posteriorly, they were numerous and coarse. There were no areas of dulness or of abnormal breath or voice sounds. The blood pressure was 138 systolic and 78 diastolic, and the heart rate was rapid. The rest of the physical examination showed nothing remarkable.

The patient was given 4 Gm. of sulfadiazine, followed by 1 Gm. every four hours for three days and, every six hours for two more days. She was digitalized over a three day period beginning October 17 and received a maintenance dose thereafter. The rest of the treatment was symptomatic and included oxygen for dyspnea and cyanosis, codeine and expectorants for cough, barbiturates for sedation and stimulants during the latter part of the course.

The temperature was irregular, rising daily to 101 F. at first and later to 102 F., with occasional higher readings. The pulse rate ranged from 100 to 120 per minute during the first five days and thereafter was about 140 except on the last day, when it rose to 160. The respiratory rate rose gradually from 20 to 40 per minute and occasionally was more rapid. Even the slightest exertion was accompanied by marked dyspnea and a sharp increase of the pulse and respiratory rates. The cyanosis had increased markedly by the sixth day in spite of oxygen. The respirations were shallow at first but later became deeper. Coarse rales extended throughout both lungs, and there were also loud, coarse rhonchi from tracheal moisture which the patient could not raise. The patient continued to feel weak and prostrated but remained mentally clear for the first five days; after that she became somewhat disoriented. On the ninth hospital day she became stuporous and later comatose. Her blood pressure fell to 70 systolic and 50 diastolic. She failed to respond to further treatment and died on October 25.

The white blood cell count was 20,100 on entry and thereafter ranged between 15,100 and 21,200, with about 90 per cent polymorphonuclear neutrophils. The hemoglobin content was 70 to 75 per cent and the red blood cell count 6,250,000. On numerous examinations the urine showed good concentration; occasional specimens showed albumin (1 plus), and all had varying numbers of white blood cells and occasional red blood cells in the sediment, and a few sulfadiazine crystals were seen on one occasion. The blood nonprotein nitrogen ranged between 30 and 40 mg. per 100 cc. except for a temporary rise to 67 mg. on October 22. On the latter date the blood carbon dioxide was reported as 68 volumes per cent. Blood sulfadiazine levels rose to 14.4 mg. per 100 cc. on October 17, then gradually dropped to 1.7 mg. by October 23, less than 1 mg. being in the conjugated form in each instance. On October 24 a lumbar puncture showed normal pressure and dynamics and yielded entirely normal fluid. Roentgenograms of the chest, taken on October 17, showed a small patch of mottled density at the base of the left lung, but subsequent ones showed finely mottled infiltration throughout both lung fields (fig. 6). Electrocardiograms, taken on October 19 and 23, showed normal axis, sinoauricular tachycardia, low T_1 and T_2 and inverted T_3 and notched T_4 waves were interpreted as consistent with myocardial disease or digitalis effect.

Repeated cultures of the sputum showed a predominance of alpha hemolytic streptococci, varying numbers of Micrococcus catarrhalis and diphtheroids and

occasional colonies of Staph. aureus. Blood cultures taken before, and on several occasions after, sulfadiazine therapy was started showed no growth. Complement fixation tests for psittacosis and Q fever, made on October 14 and again on October 24, were all negative. The titer of serum cold agglutinins, determined on October 23 and 24, was 1 : 320.

Autopsy (five hours after death).—Over the outer surface of the right upper arm there were three irregular vesicles, varying from 0.5 to 3.0 cm. in diameter, and on the left upper arm one similar vesicle. These were covered with thin brown skin and contained clear fluid.

The heart weighed 400 Gm. Beneath the endocardium of a region of the left ventricular surface of the interventricular septum were two flame-shaped hemorrhagic areas, 1 by 2.5 cm., with their apexes near the base of the aortic valve.
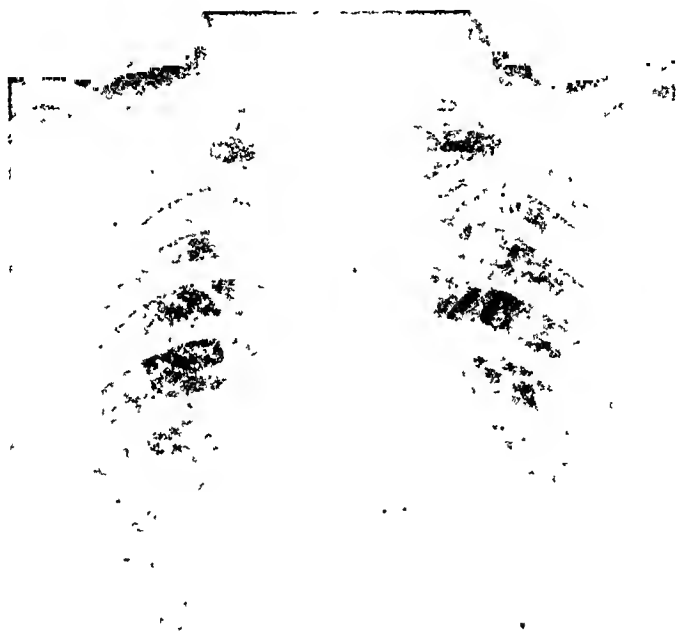
Fig. 6 (case 4).—Roentgen appearance of the lungs one week before death. There are miliary fine soft densities, more in the lower lung fields.

On section these areas were hemorrhagic and extended but a slight distance into the underlying myocardium. The heart was otherwise normal.

The left lung weighed 700 Gm.; the right, 600 Gm. Over the mediastinal surface of the lower lobe of the right lung were seen several hemorrhagic areas, about 1.5 cm. in diameter. The remainder of the surface of the lung was pink, and the lung appeared subcrepitant throughout. On section one of the branches of the pulmonary artery of the upper lobe of the right lung was found occluded by an antemortem thrombus. The lung parenchyma surrounding this vessel was darker and more hemorrhagic. The remainder of the parenchyma was pink and dry. In the left lung there were three localized areas of consolidation, two in the lower lobe and one in the upper lobe, which were wedged shaped, extending from the hilus to the periphery. On section they were raised and firm and were of the same color as the rest of the lung, which was dull pink. The consistence of the remainder of the left lung resembled that of the right.

The brain weighed 1,300 Gm. On coronal section innumerable petechial hemorrhages were found disseminated throughout the cerebral white matter. The hemorrhages were small, averaging about 1 mm. in diameter, although an occasional one measured 2 to 3 mm. in diameter. Few were situated in the cerebral cortex of the central ganglionic masses. In the pons and the cerebellum there were scattered hemorrhages throughout the white matter, but in the medulla few were seen.

Microscopic Examination.—Some alveoli of the upper. lobe of the right lung contained albuminous precipitate and red cells; others, polymorphonuclear leukocytes, and still others, varying numbers of polymorphonuclear leukocytes, mononuclear cells and desquamated alveolar lining cells. Some of the latter contained phagocytosed polymorphonuclear leukocytes. There was an occasional multinucleated cell, apparently of alveolar lining cell origin. In some of the alveoli the lining cells were swollen. Some alveoli .contained a hyaline membrane. There was a perivascular infiltration of plasma cells and some lymphocytes. A small artery contained an antemortem thrombus with a subendothelial infiltration of monocytes and plasma cells adjacent to it. Many alveoli in sections from the lower lobe of the right lung contained red cells, polymorphonuclear leukocytes and numerous cocci. In some alveoli the lining cells were swollen and desquamated, and vacuolated cells were seen in the lumens. An occasional bronchiole showed beginning organization of the exudate within the lumen. There was a perivascular infiltration of lymphocytes and plasma cells. Many alveoli were essentially normal.

The picture varied greatly from section to section of the left lung. In some the alveoli showed no change. In others they contained numerous red blood cells. Still other alveoli were filled with desquamated lining cells and rare giant cells. One section contained an abscess' in which there were masses of cocci. Elsewhere other alveoli contained numerous polymorphonuclear leukocytes and some fibrin. The lining cells of many alveoli were swollen. In still other regions extensive organization of the alveolar exudate was taking place. The bronchioles as a rule were empty, but a few contained mucus, scattered polymorphonuclear leukocytes and some red blood cells. An occasional bronchiole showed organization within its lumen. There was a perivascular infiltration of plasma cells and some lymphocytes and a similar but less striking infiltration of the walls of some bronchioles. A rare blood vessel contained a thrombus.

In the heart there were subendocardial hemorrhages extending into the adjacent myocardium, with necrosis of a few muscle fibers immediately adjacent to the hemorrhages. The spleen was congested,. and the pulp contained numerous polymorphonuclear leukocytes, scattered plasma cells and stem cells. A section from the skin showed a vesicle which was filled with albuminous precipitate. The overlying. epidermis was necrotic in its deeper portions. The base of the vesicle was made up of connective tissue.

There were widely disseminated lesions in the white matter of the cerebrum and the brain stem. They consisted of small foci of demyelination and proliferation of microglial phagocytes. Some of the lesions were surrounded by extravasated red cells and ring hemorrhages without microglial proliferation (fig. 7). The hemorrhages seemed to be more recent than the proliferative lesions. Many of the lesions were perivenous, but in others a centrally placed blood vessel could not be identified. There were no lymphocytes infiltrating the Virchow-Robin spaces or the leptomeninges. Nerve cells were preserved, even those adjacent to the lesions. No thrombosed vessels were seen.

Bacteriologic Study.—Cultures of the heart's blood yielded no growth. From the upper lobe of the left lung hemolytic Staph. aureus, a streptococcus with alpha

hemolysis and Eberthella coli and Clostridium welchii were isolated. Attempts to isolate a virus from suspensions of the lung were unsuccessful.

CASE 5 (courtesy of Drs. Leroy E. Parkins and Shields Warren).—A 40 year old white married woman was admitted to the Baptist Hospital on Oct. 22, 1942. She first became ill on October 12 with coryza, a raw feeling of the throat and some cough. Her physician found her to be acutely ill, with fever, diffusely injected throat and mucoid nasal discharge. She was given sulfadiazine, 1 Gm. every four hours, and improved temporarily, but her symptoms rapidly returned and became worse. On October 19 a few rales were heard over the lower lobe of the right lung.
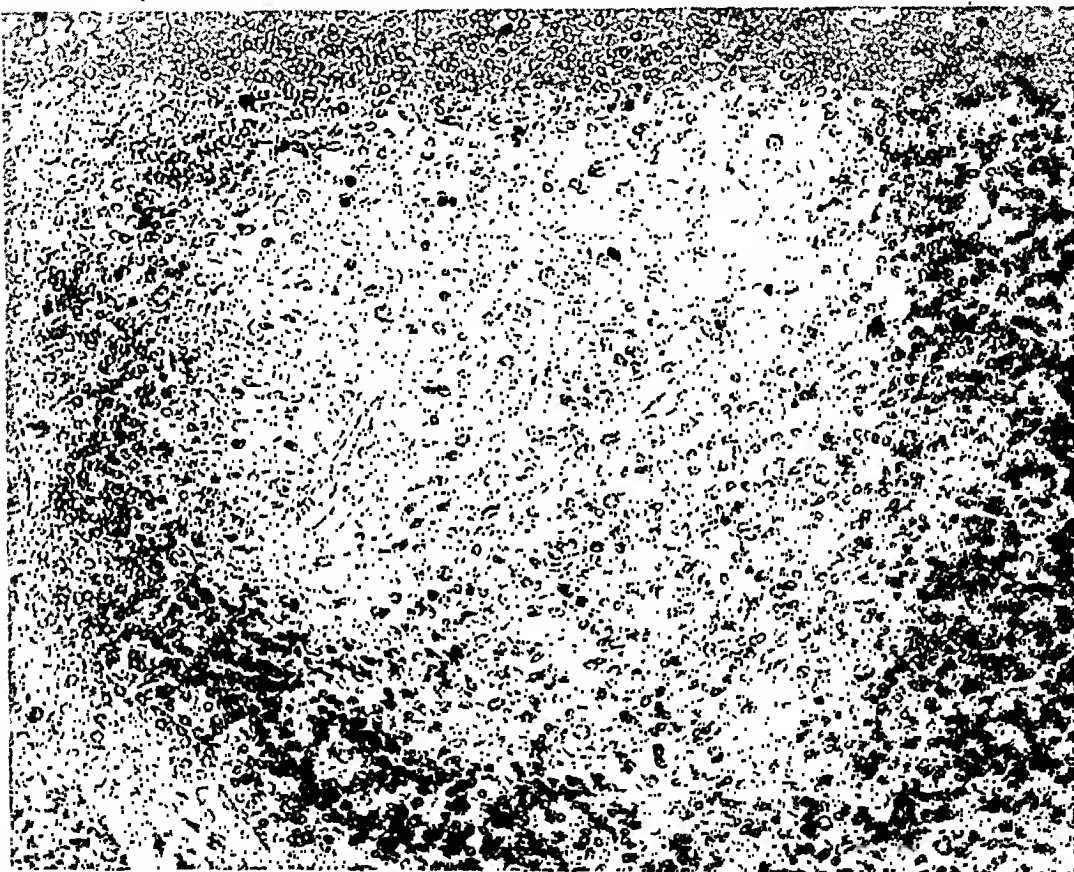
Fig. 7 (case 4).—Brain; perivascular hemorrhages and glial proliferation. Phloxine–methylene blue stain; × 350.

On admission she was extremely ill, with marked dyspnea and some stridor. Her respirations were "moist" and noisy. There was patchy exudate on the tonsillar crypts, as well as a thin gray membrane on the soft palate and the tongue. Throughout both lungs there were showers of rales. The temperature ranged mostly between 102 and 104 F.: the pulse rate was about 120 per minute during the first four days and 140 thereafter, and the respiratory rate rose steadily from 30 to about 60 per minute. The white blood cell count was 12,700, with 82 per cent polymorphonuclear neutrophils on admission; 11,600 on October 25, and 24,000 on October 27. The hemoglobin was 90 per cent and the red blood cell count about 4,500,000 on three occasions. The urine revealed a slight trace of albumin

and no other abnormalities. The blood nonprotein nitrogen was 36 mg. per 100
cc., and the sulfadiazine levels were about 6 mg. per 100 cc. Cultures of muco-
purulent sputum showed no significant organisms. A blood culture made on entry
was negative. Roentgenograms of the chest on admission showed areas of slightly
increased density in the lower lobe of the left lung and some in the lower lobe
of the right lung. This density increased in extent and appeared more mottled
in successive films until there was miliary mottling involving all of both lung
fields (fig. 8).

The administration of sulfadiazine and alkalis was discontinued after three days.
The patient was kept in an oxygen tent and given barbiturates for restlessness,
codeine for cough, and parenteral fluids. Dyspnea, cyanosis, restlessness, sweating
and weakness increased progressively. Bubbling rales were now heard in the
chest, and the patient became disoriented and irrational. Later abdominal disten-
tion developed, with slight edema of the buttocks, and the patient died on
November 1.
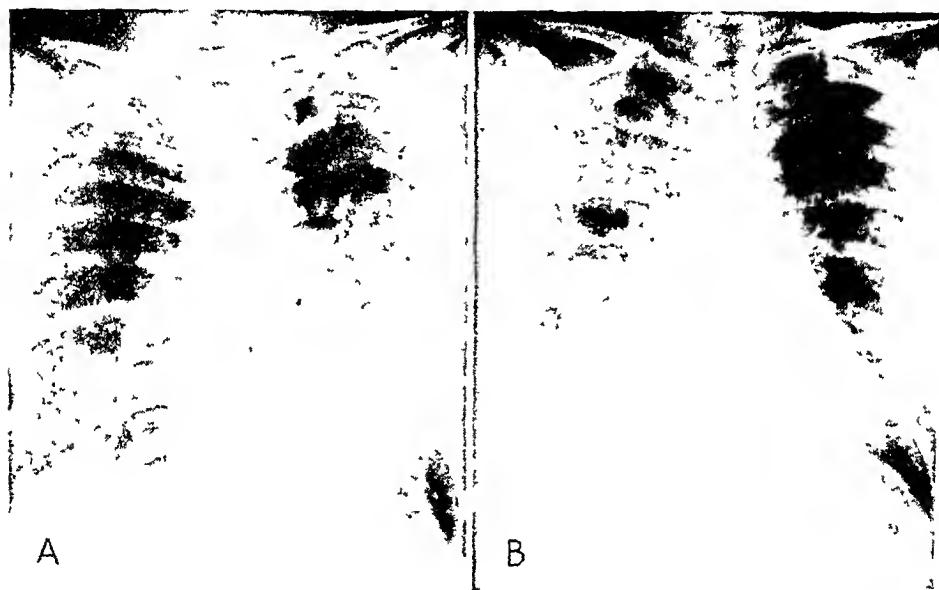
Fig. 8 (case 5).—A, roentgenogram taken three days after admission, showing
nodular and confluent densities in the left lung field and softer densities extending
down and out from the right hilus.

B, roentgenogram taken two days later, showing extension of the lesions to
involve most of the right lung and more discrete lesions over the previous area of
density on the left.

Autopsy (one and one-half hours after death).—The left lung weighed 500
Gm. and the right 850 Gm.; both were dull deep reddish gray except for the
upper lobe of the left lung, which was light gray. The lower lobe of the left
lung was much darker than the other lobe. Palpation of the lungs revealed numer-
ous scattered areas, averaging 3 to 4 cm. in diameter, which were much firmer
than normal. Sections through the lungs showed moderately firm, dense, red,
deeply hyperemic tissue with numerous darker nodular areas throughout all lobes
except the left upper lobe, which showed considerable edema. The lung tissue as
a whole was firmer than normal, but this change varied from place to place. The
firmness was most marked in the lower lobe of the left lung and was generally

present throughout the right lung as well. The bronchi and bronchioles showed slight hyperemia and had a small amount of mucus within the lumens.

Microscopic Examination.—In sections from the lungs some alveoli were filled with edema fluid, some with red blood cells and others with an exudate made up almost entirely of mononuclear cells and desquamated alveolar lining cells (fig. 9 *A*). Many of the latter contained phagocytosed material. Other alveoli were filled with polymorphonuclear leukocytes and some with fibrin; this was especially true of the alveoli surrounding bronchioles, which contained a similar exudate. The alveolar lining cells were swollen in many areas, and in these cells mitotic figures were not infrequent (fig. 9*B*). A considerable number of alveoli contained a hyaline membrane. In sections from the lower lobes organization of the alveolar exudate was taking place in many areas. In others the connective tissue of the alveolar walls appeared to be increased. The majority of the bronchioles had little exudate in their lumens, but some did contain numerous polymorphonuclear leukocytes, a few large mononuclears and mucus. There was a rare small focus of necrosis of the lining epithelium. The peribronchiolar and perivascular tissues were infiltrated by plasma cells, lymphocytes, large mononuclears and a few polymorphonuclear cells. A few arterioles contained thrombi.

In the pulp of the spleen there were numerous polymorphonuclear leukocytes and plasma cells and, in addition, a rare myelocyte; also, there was a considerable number of macrophages, many of which contained red blood cells and some of which had phagocytosed various types of leukocytes. Several veins showed a subendothelial infiltration of lymphocytes, plasma cells and an occasional eosinophil. In the liver there were scattered necrotic liver cells; some of these had been completely removed, and the spaces they formerly occupied contained polymorphonuclear leukocytes and occasional large mononuclear cells. The Kupffer cells were prominent; some had phagocytosed red blood cells, others leukocytes. The portal connective tissue was infiltrated by lymphocytes and plasma cells.

CASE 6.—This case, too, has been reported in detail elsewhere.[8] The patient was a 24 year old foundry worker whose illness began Dec. 26, 1942 with a shaking chill, high fever, substernal pain and cough, followed in two days by soreness of the mouth and throat and dysphagia. Sulfonamide compounds were then given, without effect, and the patient complained of headache, photophobia, dysuria, bloody sputum and a vesicular rash over the entire body, all of which progressed until he entered the hospital on December 31. He had had similar skin eruptions three times in the previous ten years, and he also frequently had diffuse erythema following ingestion of certain foods. In the hospital he was found to have generalized erythema multiforme exudativum involving the skin, the mucous membranes, the genitals and the conjunctivas and diffuse atypical pneumonia. These all progressed until the patient died on Jan. 18, 1943.

The white blood cell count was 12,250 at first and then dropped to between 3,100 and 6,200, with 91 to 80 per cent polymorphonuclear neutrophils. Urinalyses showed small amounts of albumin, occasional white blood cells and many red blood cells. Roentgenograms showed increasing areas of mottled infiltration of both lungs. Sputum smears showed polymorphonuclear leukocytes and few organisms, and cultures showed only a few alpha hemolytic streptococci at first but later yielded increasing numbers of hemolytic staphylococci (Staph. aureus) and hemolytic streptococci. The blood cultures were all negative. Repeated complement fixation tests for psittacosis were negative. The cold agglutinin titers on January 2, 4, 11, 15 and 18 were $< 1:4$, $< 1:4$, $1:8$, $1:32$ and $< 1:4$, respectively.
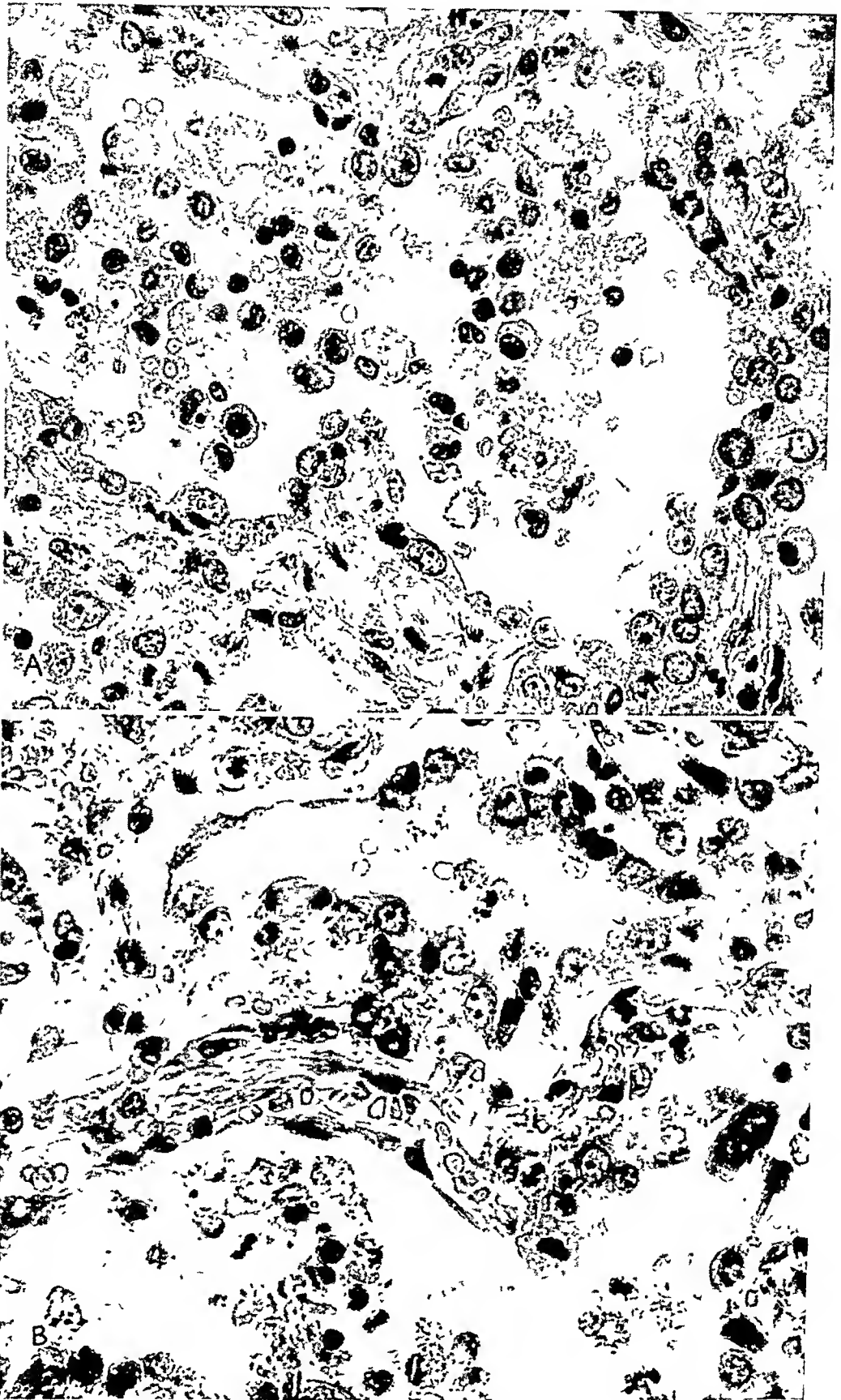
Figure 9
(See legend on opposite page)

Autopsy (fifteen hours after death).—The skin, the oral mucous membrane, the scrotum and the penis showed vesicles and bullae in various stages, some intact, others dried up, encrusted, hemorrhagic or ulcerated. The conjunctivas were markedly injected.

The pleural surfaces were covered with a thin layer of fresh fibrin. The left lung weighed 1,145 Gm.; the right, 1,675 Gm.; both were firm, mostly subcreptitant, with some noncrepitant areas, and their surfaces were deep blue-red and appeared hemorrhagic posteriorly. The cut surfaces oozed a large amount of blood and showed miliary nodules which were yellow-white against a blue to gray-red background and which appeared purulent, but no pus could be expressed. A small amount of pus could be expressed from the smaller bronchioles. The mucosal surfaces of the trachea and bronchi were hyperemic and covered with a slimy dark red exudate containing much blood. The tracheobronchial lymph nodes measured about 2.5 cm. in diameter.

Microscopic Examination.—In sections from several lobes of the lungs the majority of the alveoli contained desquamated alveolar lining cells and large mononuclear cells; the alveolar lining cells were swollen, and varying numbers of mitotic figures were seen. Some alveoli were empty and markedly distended; others contained albuminous precipitate; occasional ones showed hyaline membrane formation; still others contained some polymorphonuclear leukocytes, and many contained fibrin in which there were masses of cocci. The bronchioles contained polymorphonuclear leukocytes, large mononuclear cells and cocci. There was a marked perivascular and peribronchiolar infiltration of plasma cells and some lymphocytes and there were many plasma cells in the alveolar walls and also in their lumens (fig. 10). The septums showed edema. In sections from the upper lobe of the left lung some alveoli contained masses of fibrin, some of which was undergoing organization. Sections from the lower lobe of this lung also showed an area of extensive abscess formation with an exudate of polymorphonuclear leukocytes and many cocci. Adjacent to this area was a focus of gangrene in which numerous cocci and bacilli were present. One bronchiole had lost its epithelium, and its denuded surface was covered with polymorphonuclear leukocytes and fibrin. The pleura was covered with a thin layer of fibrin. The trachea was congested, and the submucosa and the region around the glands was infiltrated by plasma cells.

A section of the skin showed a vesicle containing an albuminous precipitate, some fibrin and a few polymorphonuclear leukocytes. The cells of the inferior surface of the epidermis were necrotic and invaded by polymorphonuclear and large mononuclear cells. The base of the vesicle consisted of the connective tissue of the corium; it was covered with fibrin and showed a perivascular infiltration of lymphocytes and occasional plasma cells. There was a similar infiltration about the coil glands. The cerebral meninges contained a few lymphocytes, large mononuclears and plasma cells. Some of the other organs were infiltrated by similar cells.

Fig. 9 (case 5).—*A,* swollen alveolar lining cells. Alveolar exudate of desquamated alveolar lining cells and large mononuclears. Phloxine–methylene blue stain; × 650.

B, swelling of alveolar lining cells, two of which contain mitotic figures. Giemsa's stain; × 850.

Bacteriologic Study.—Cultures of both upper lobes yielded beta hemolytic streptococci and hemolytic Staph. aureus. Attempts to isolate a virus from vesicle fluid and from a filtered suspension of lung were unsuccessful.

CASE 7.—A 35 year old Jamaica Negro welder entered the Boston City Hospital on Jan. 12, 1943 because of increasing cough and dyspnea. On Dec. 27, 1942 he began to feel tired, lost his appetite and had generalized aches and pains. He worked during the next two days, but on December 30 a severe nonproductive cough developed, associated with some soreness of the chest. On January 1 he raised a small amount of bloody sputum, a culture of which showed no pathogenic organisms. His physician noted diffuse rales in his lungs, diagnosed "virus pneumonia" and prescribed sulfonamide drugs, of which the patient took 1 Gm. every two hours at first and then 1 Gm. every four hours until January 9. During this time his cough and substernal pain increased and he became progressively more dyspneic and cyanotic until he was admitted to the hospital. He had previously been in good health except for attacks of lead poisoning in 1928 and 1929.
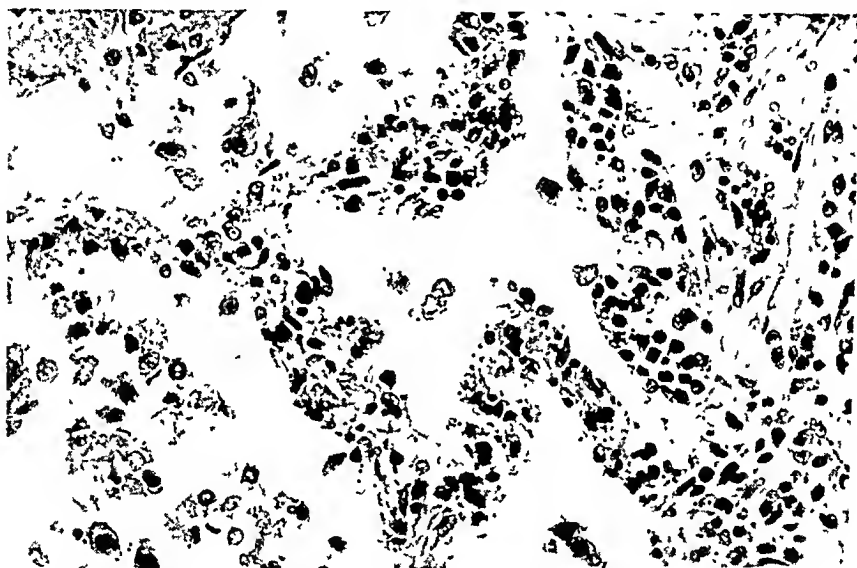
Fig 10 (case 6).—Alveolar walls infiltrated by plasma cells. Phloxine-methylene blue stain; × 225.

On entry the patient was markedly dyspneic and moderately cyanotic. His respirations were short, grunting, labored and accompanied by some wheezing. His face was flushed and covered with cold sweat. The skin was warm over the trunk and cold over the extremities. The lips and the nail beds were cyanotic. The temperature was 101 F., the pulse rate 138, the respiratory rate 48 and the blood pressure 155 systolic and 95 diastolic. The tonsillar crypts were covered with white exudate, and the vessels of the posterior cavity of the pharynx were injected. There was some dulness of the right upper anterior region of the chest, and some ill defined areas of dulness were present in both lungs posteriorly. Loud musical and crepitant rales were heard throughout both, lungs, but there were no definite changes in the breath and voice sounds.

The white blood cell count was 29,800 on admission and 50,000 on the following morning. Practically all of the cells were polymorphonuclears. The urine was normal. A blood culture made on entry showed no growth. The sputum was purulent and slightly blood streaked. Stained smears showed polymorphonuclear

leukocytes, a few epithelial cells, occasional red blood cells and a few gram-positive diplococci and rare short gram-negative rods. Cultures yielded a few alpha strep-tococci, a few staphylococci and a few H. influenzae. The blood nonprotein nitro-gen was 38 mg. per 100 cc. The Hinton test of the blood was negative. The psittacosis complement fixation test of a serum specimen taken January 11 was negative. The cold agglutinin titers, determined on January 11 and 13, were 1:40 and 1:160, respectively.

Sulfapyrazine was administered, 4 Gm., followed by 1 Gm. every four hours to a total of 9 Gm. Fluids were given orally. Oxygen was administered by nasal catheter. The patient's dyspnea and cyanosis, however, increased progressively. On the morning after admission he became excited and suddenly jumped out of bed against the resistance of an attending nurse. He collapsed in the middle of the ward and immediately stopped breathing.

Autopsy (fourteen hours after death).—The apex of the right lung was attached to the chest wall by fresh fibrinous adhesions, which were yellow and easily broken. Elsewhere the surfaces were smooth and glistening. The pericardial cavity contained 40 cc. of thin yellow fluid with a faint bloody tinge. The heart weighed 410 Gm. and showed no abnormalities.

The left lung weighed 740 Gm.; the right, 750 Gm.; both were subcrepitant throughout. On section the cut surfaces oozed a large amount of blood and were rough and finely nodular. These nodules were made up of small pink-white raised areas surrounding bronchioles and measuring 1 to 1.5 mm. in diameter. Otherwise the surface was bright red-gray. Numerous small hemorrhagic areas were present in all lobes except the middle lobe of the right lung. An occasional bronchiole contained yellow purulent material. The mucosa of the bronchi and trachea was pink and velvety. The tracheobronchial lymph nodes were enlarged, measuring 2 cm. in diameter.

The brain showed marked congestion of the meningeal vessels. No other gross abnormalities were made out.

Microscopic Examination.—Sections from the upper and lower lobes of the right lung showed essentially the same picture. Some alveoli contained albuminous precipitate, some polymorphonuclear cells and a moderate amount of fibrin. Some alveoli were distended and lined with a hyaline membrane. In a few places the alveolar lining cells were swollen, and some had desquamated and had surrounded masses of fibrin. The alveolar walls in foci contained fibrin, polymorphonuclear leukocytes and plasma cells. The bronchioles were filled with polymorphonuclear cells. There was a peribronchiolar and perivascular infiltration of plasma cells. The septums were edematous, and the septal lymphatic channels were filled with plasma cells, some polymorphonuclear leukocytes and red blood cells. The great majority of the alveoli of the upper lobe of the left lung contained precipitated albumin. There was a rare small bronchiole filled with polymorphonuclear leuko-cytes, as were the adjacent alveoli. One large bronchiole had numerous plasma cells and a few lymphocytes infiltrating its wall. In the lower lobe of the left lung there was rather extensive organized pneumonia and beginning organization of the exudate of the bronchioles. The alveoli otherwise were unchanged or contained an albuminous precipitate except in places where the alveoli were lined with cuboid cells, and here their lumens contained desquamated alveolar lining cells, which were often vacuolated. The bronchiolar walls were infiltrated by numerous plasma cells. There was edema of the septums.

The heart had a focal infiltration of a few large mononuclears, plasma cells and mast cells in the interstitial tissue of the myocardium. The spleen contained

an increased number of plasma cells in the pulp and also macrophages, some vacuolated and others containing hemosiderin. The liver showed scattered small areas where the hepatic cells had disappeared, and there were a few lymphocytes, plasma cells and occasional polymorphonuclear leukocytes infiltrating such areas. The testis showed markedly decreased spermatogenesis. In a section from a mediastinal lymph node the sinuses contained many macrophages, some plasma cells and polymorphonuclear leukocytes. There was marked hyperplasia of both the red and the white series of the bone marrow. Sections of the brain revealed no abnormality.

Bacteriologic Study.—Culture of heart's blood showed no growth. Cultures of material from the lower lobes of the lungs yielded a streptococcus with alpha hemolysis.

CASE 8.—A 44 year old mother of two children was known to have had rheumatic heart disease since childhood and during the past eight years had repeated bouts of dyspnea and edema of the ankles, for which she had been taking digitalis regularly. She had been working hard as a waitress when, Oct. 29, 1943, she had a sudden onset of chilliness and malaise, and her temperature rose to 104 F. A severe cough developed, productive of "rusty" sputum, and the patient became dyspneic. A physician diagnosed bilateral lobar pneumonia and prescribed sulfathiazole, 1 Gm. every four hours at first and then 0.5 Gm. every four hours. This therapy was discontinued after two days because of poor urinary output. Fever and symptoms continued and progressed until she was admitted to the Boston City Hospital on November 5.

When the patient entered the hospital, she was moderately dyspneic and stuporous. There were dulness and diminished breath sounds over the lower lobe of the left lung posteriorly, with some wheezing in that area, and showers of crepitant rales throughout both lungs. The heart was moderately enlarged. The sounds were of good quality but grossly irregular, and there were signs of well advanced mitral stenosis. The blood pressure was 110 systolic and 60 diastolic. The abdomen was tympanitic and distended, and there was tenderness in the right upper quadrant, but the liver and the spleen were not felt, and there was no peripheral edema.

Sulfapyrazine was administered, 3 Gm. on admission and 1 Gm. every six hours thereafter. The patient also received a maintenance dose of digitalis, aminophylline for wheezing, and barbiturates and dihydromorphinone hydrochloride ("dilaudid hydrochloride") for sedation and restlessness. Fluids were given by mouth and by hypodermoclysis. Oxygen was given by tent and later was mixed with helium and administered under positive pressure, without relief. The temperature ranged between 102 and 104 F., the pulse rate between 100 and 120 and the respiratory rate between 30 and 45. The musical and coarse rales increased throughout both lungs, but no definite signs of consolidation were made out. The patient became progressively dyspneic and stuporous and died on November 10.

The white blood cell count on entry was 12,400, with 80 per cent polymorphonuclears, and thereafter the counts ranged between 11,400 and 14,200. Several urine specimens ranged in specific gravity from 1.022 to 1.030; all showed albumin (3 plus) and occasional white blood cells. The blood nonprotein nitrogen was 28 mg. per 100 cc. on admission and rose progressively to 56 mg. on November 8 and to 72 mg. on November 10. Daily determinations of sulfapyrazine level showed a progressive rise from 3 to 10 mg. per 100 cc., of which from 0.5 to 4 mg., respectively, were in the conjugated form. Roentgenograms of the lungs, November 6, revealed irregular infiltration of the left lower and the right middle lung field with enlargement of the heart, which showed the typical rheumatic deformity. Some extension of the pulmonary infiltrations was seen in the film of November 9.

An electrocardiogram taken on admission showed auricular fibrillation. Several specimens of sputum were all rusty, showed numerous polymorphonuclears and red blood cells and rare gram-positive extracellular diplococci. Sputum cultures showed alpha hemolytic streptococci at first, but hemolytic staphylococci (Staph. aureus) appeared in great numbers on November 10. Blood cultures, made on entry and again on November 10, showed no growth. The titer of cold agglutinins, November 9, was 1:80.

Autopsy (eighteen hours after death).—The right pleural surfaces were smooth and glistening. The left pleural cavity was completely obliterated by old fibrous adhesions. The pericardial cavity contained 200 cc. of clear amber fluid. The heart weighed 550 Gm. The chordae tendineae of the mitral valve were shortened and thickened. The mitral valve was thickened, with marked interadherence of the cusps. Before being opened the valve admitted the tip of the finger only. The aortic valve showed a slight degree of roughening. In the left auricle there was an organizing thrombus.
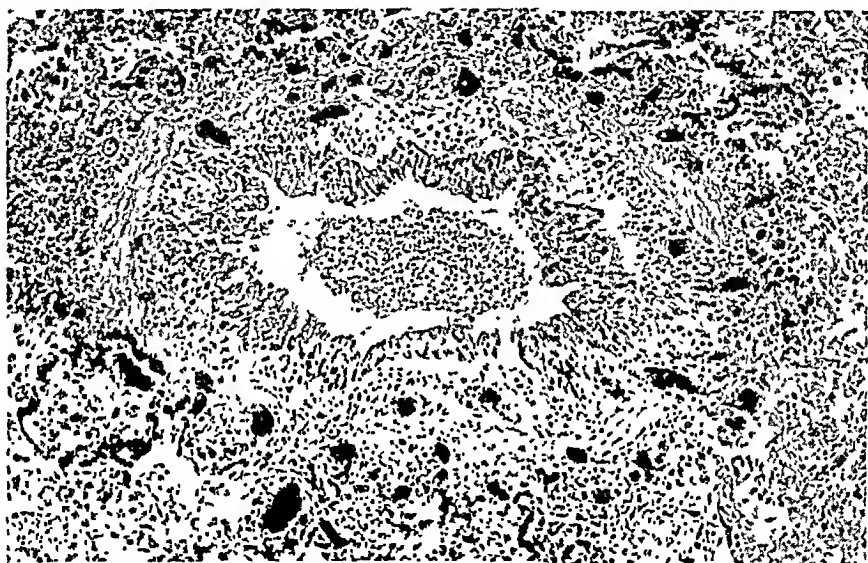
Fig. 11 (case 8).—Lesion of bronchiole similar to that shown in figure 2. The epithelium is intact and increased in thickness. Phloxine–methylene blue stain; × 100.

The left lung weighed 750 Gm.; the right, 950 Gm. The apexes were brown and crepitant, and the bases were congested and edematous. The lower lobe of the right lung had a small amount of pus in the bronchioles. Elsewhere there was no evidence of exudate in the bronchioles, the bronchi or the trachea.

Microscopic Examination.—Some alveoli of the upper lobe of the right lung contained desquamated alveolar lining cells; others, similar cells, monocytes and polymorphonuclear leukocytes. In a few alveoli there were clumps of fibrin surrounded by mononuclear cells. The alveolar lining cells, as a rule, were swollen. The alveolar walls were infiltrated in places by plasma cells and polymorphonuclear leukocytes. The bronchioles contained mucus and a few polymorphonuclear cells. There was a peribronchiolar and perivascular infiltration of a moderate number of plasma cells, some polymorphonuclear leukocytes and an occasional mast cell. The septums were edematous. In the lower lobe of the right lung some alveoli were empty and distended, while others contained an albuminous precipitate,

desquamated alveolar lining cells and large mononuclear cells. The great majority contained numerous mononuclear cells, and in many places a varying number of polymorphonuclear leukocytes as well. A few alveoli contained fibrin surrounded by mononuclear cells. In many alveoli the alveolar lining cells were swollen. The alveolar walls were infiltrated by plasma cells and in foci also by polymorphonuclear leukocytes. The bronchioles contained mucus, some mononuclear cells and polymorphonuclear leukocytes. There was a perivascular and peribronchial infiltration of a moderate number of plasma cells and some polymorphonuclear leukocytes, and the bronchiolar epithelium was thickened (fig. 11). Several blood vessels contained thrombi. A Lee-Brown stain showed reduplication of the capillaries but slight if any thickening of the capillary basement membrane. The lower lobe of the left lung was similar to that of the right lung.

The heart had several rather large scars in the myocardium. The liver showed . an occasional necrotic cell invaded by polymorphonuclear leukocytes.

Bacteriologic Study.—Cultures of the heart's blood and of the upper and lower lobes of the right lung showed no growth. A streptococcus with alpha hemolysis was cultured from the lower lobe of the left lung.

SUMMARY OF CLINICAL FINDINGS (TABLE)

Of these 8 cases, 7 occurred in the latter months of 1942, and 1 occurred the following year. Three of the patients were males, aged 17, 24 and 35 years, and the others were females between 40 and 53 years old.

The illness, in these cases, was characteristic of the severe and extensive type of primary atypical ("viral") pneumonia.[1] It began either with general malaise, "grippy" feeling and fever or more abruptly with chills or chilly sensations. These were followed by cough, which was productive of scant mucoid and sometimes blood-streaked sputum and was accompanied by pain or soreness of the anterior part of the chest. The course was characterized by increasing dyspnea and cyanosis, then air hunger and delirium, sometimes by stupor and coma terminally. In 2 cases there was a diffuse bullous type of erythema multiforme and in 2 others exudative pharyngitis. In 1 case there was rheumatic heart disease with mitral stenosis, but the heart was compensated at the time of the onset of pneumonia.

In the lungs the characteristic signs were the increasing numbers of crepitant rales, which were eventually heard throughout; there were only transient areas of dulness posteriorly, with either suppression or increase of breath sounds, but no persistent areas of consolidation. Musical rales or wheezing were heard only occasionally in some cases, and loud rhonchi were heard terminally. There was no improvement from sulfonamide drugs, and oxygen gave only partial, if any, relief in the later stages.

There was slight to marked polymorphonuclear leukocytosis, and in only 2 cases was leukopenia noted during part of the course. The red blood cell counts were normal except in the 2 cases in which acute

Certain Relevant Data in Eight Fatal Cases of Primary Atypical ("Viral") Pneumonia

	Case 1 Male, 17	Case 2 Female, 50	Case 3 Female, 53	Case 4 Female, 52	Case 5 Female, 40	Case 6 Male, 24	Case 7 Male, 35	Case 8 Female, 11
Sex and age................								
Dates:								
Admission............	8/26/42	9/30/42	10/15/42	10/14/42	10/22/42	12/31/42	1/12/43	11/6/43
Onset................	8/12	9/27	10/6	9/25	10/12	12/30	12/27/42	10/29
Death................	9/3	10/18	10/19	10/25	11/1	1/18/43	1/13/43	11/10
White blood cell counts (×1,000).............	12.2-17.5	4.7-32.6	28.3-57.0	15.7-21.2	11.6-24.1	12.3-3.1	29.8-50	11.4-14.2
Polymorphonuclears, %....	60-85	68-96	77-94	90	82	91-80	92-98	80
Bacteriologic findings* in:								
Blood..............	0	0	0	0	0	0	0	0
Sputum..............	Streptococcus with alpha hemolysis; Staph. aureus	Staph. aureus	......	Streptococcus with alpha hemolysis; Staph. aureus	Streptococcus with alpha hemolysis	Streptococcus with alpha hemolysis; Staph. aureus	Streptococcus with alpha hemolysis; Staph. aureus; H. influenzae	Streptococcus with alpha hemolysis; Staph. aureus
Cardiac blood (autopsy)..	0	0	......	0	......	0	0	0
Lungs (autopsy)...........	Staph. aureus	Streptococcus with alpha hemolysis; Staph. aureus; H. influenzae	......	Staph. aureus; streptococcus with alpha hemolysis; E. coli	......	Streptococcus with beta hemolysis; Staph. aureus	Streptococcus with alpha hemolysis; Staph. aureus	Streptococcus with alpha hemolysis †
Serologic tests* for:								
Psittacosis, complement fixation titer.............	1:256	0	0	0	......	0	0	......
Q fever, complement fixation titer............	......	0	......	0	......	0	......	......
Cold agglutinins..........	1:640	1:160 to 1:1,280	+ (?) ‡	1:320	......	<1:4 to 1:32	1:40 to 1:160	1:80
Virus isolated............	0	0	0	0	......	0	0	......
Complications............	Erythema multiforme exudativum	Hemolytic anemia	Hemolytic anemia	......	......	Erythema multiforme exudativum	......	Mitral stenosis
Sulfonamide therapy, days....	9	11	5	5	±18	3	9	2

* A zero indicates that the cultures (or tests) were negative; a blank space, that no cultures (or tests) were made.

† This was from one lobe only, two other lobes yielded no growth.

‡ Tests were not done, but hemolytic anemia and difficulty of cross matching for transfusion suggest that cold agglutinins were present.

603

hemolytic anemia was present.[4]   Blood cultures were all negative. Sputum smears showed polymorphonuclear leukocytes and few organisms, and cultures showed predominantly alpha hemolytic streptococci, and most of them later yielded staphylococci.   There were also hemolytic streptococci and Hemophilus influenzae in 1 case each.   Cold agglutinins were found in moderate or high titer in all of the 6 cases in which serum was tested.   A significant titer of complement fixation for psittacosis was found in only 1 of 5 cases, and the tests for Q fever were negative.   In every case roentgenologic examination revealed a characteristic soft miliary type of density, which eventually spread to involve most of the lung fields, and in some cases there were more diffuse areas of density that probably represented transient atelectasis.

The duration of the disease from the time of the first symptom was thirteen days in 2 cases, thirty-one days in 1 case and seventeen to twenty-four days in the remaining 5 cases.

SUMMARY OF THE PATHOLOGIC OBSERVATIONS

Grossly, the lungs were enlarged and increased in weight.   They presented an appearance of congestion, and there were scattered areas in which crepitus was diminished or absent especially in the posterior and inferior portions.   The surfaces overlying the areas of atelectasis were sharply outlined and dark purple-red.   The upper and anterior portions were more crepitant, and here the surfaces were more grayish white. There was some scattered fibrinous exudate on the surface in half of the cases.   The cut surfaces presented a characteristic miliary nodular appearance, most impressive in the lower lobes, the nodules varying from 1 to 2.5 mm. in diameter, usually appearing grayish white to yellowish against a deep hemorrhagic background.   No exudate could be expressed from the nodules; bloody fluid was expressed from the surrounding surface, and only small amounts of mucopurulent materials were expressed from some of the bronchioles.   The mucosa of the trachea and the bronchi was generally hyperemic and covered with dark mucus.

The characteristic histologic features, in these cases of primary atypical pneumonia, were the nature of the alveolar exudate, the swelling. and proliferation of the alveolar lining cells and the interstitial infiltration.

The alveolar exudate was made up primarily of mononuclear cells. These cells appeared to be of two types—desquamated alveolar lining cells, and large mononuclear cells.   The alveolar lining cells were the largest cells.   They had a round nucleus and abundant acidophilic cytoplasm, which was sometimes vacuolated.   The cells sometimes contained phagocytosed material of various sorts, such as carbon and nuclear

---

4. Finland, M.; Peterson, O. L.; Allen, H. E.; Samper, B. A., and Barnes, M. W.: J. Clin. Investigation **24:**458, 1945.

débris. Mitotic figures occurred in these cells but were rare. As a rule, the desquamated alveolar cells had a single nucleus, but two or more were occasionally seen. The large mononuclear cells were medium sized, with a round or an indented nucleus. Their cytoplasm was acidophilic and not infrequently contained phagocytosed material. Plasma cells also occurred in some instances. In addition to this cellular exudate, varying amounts of edema fluid, red blood cells and fibrin were present.

The swelling of the alveolar lining cells was a prominent feature. The cytoplasm of such cells tended to be basophilic. Mitotic figures were not infrequent. The shape of these cells was often oval, and their appearance differed markedly from the cuboid type of lining cell seen in other conditions, such as fibrosis of the alveolar walls associated with heart disease, healed tuberculosis, staphylococcic peneumonia or complicating influenza or any other condition which leads to scarring. Necrosis of the alveolar lining cells was not seen; so presumably their swelling, proliferation and desquamation were a reaction to the presence of the causative agent.

The interstitial infiltration was a constant and conspicuous feature. This infiltration occurred in the walls of the bronchioles and around the blood vessels and frequently extended into the walls of the alveoli. The cellular components of this infiltrate were for the most part plasma cells, some of which were immature. Other types of cells present were lymphocytes and mast cells.

The bronchioles, with a rare exception, showed no evidence of injury and contained no exudate except for those in areas where a secondary bacterial infection was present. In these areas the lumens of the bronchioles contained numerous polymorphonuclear leukocytes and often some mucus and, in addition, cocci. In some but not all of the cases in which such secondary infection was present, the alveoli also contained polymorphonuclear leukocytes and sometimes fibrin.

Fibrinous pleuritis of any significant extent occurred only in the presence of secondary bacterial invaders.

Edema of the septums was also a prominent feature, and the septal lymphatic channels often were filled with a cellular exudate made up of plasma cells and various types of leukocytes.

Thrombi were a frequent finding, especially in the smaller arteries and veins. The cause of the formation of such thrombi was not entirely clear, since they were by no means always associated with a contiguous acute inflammatory lesion. The vessel walls showed no visible lesions aside from an occasional subendothelial cellular infiltration. Some of these thrombi had become endothelized and some had undergone partial organization.

Hyaline membranes were found in the alveoli in approximately one half of our cases.

Organization of the alveolar and bronchiolar exudates was found in the majority of the cases but was not extensive.

The only lesions of note occurring in other organs were found in the liver and the brain. There was some necrosis of the hepatic cells, involving only single cells or a few cells; in no case was it extensive. Whether these lesions can be attributed to the etiologic agent of atypical pneumonia, it is impossible to say, since in all our cases the respiratory system was secondarily infected by bacteria to some extent. The lesions of the brain consisted of perivascular hemorrhages with some glial proliferation and were conspicuous in only 1 case.

COMMENT

The pathologic changes observed in our cases correspond with those described by other authors.[2] Longcope [2a] felt that, while the lesions are not unique, they are distinctive and are entirely different from those of pneumococcic pneumonia and lobular pneumonia due to common micro-organisms. The pathologic changes as a whole appear to be more like those of psittacosis [5] than those of any other disease. However, the lesions are not identical in that the alveolar exudate of psittacosis contains considerably more fibrin and red blood cells and interstitial infiltration is by no means so conspicuous a feature as in these cases of primary atypical pneumonia.

The possibility that case 1 was indeed one of psittacosis must be considered because of the high titer of complement-fixing antibodies in the serum. There was no history of the patient's having been exposed to birds in this case, and the pathologic changes noted in the lungs were essentially the same as those in the other cases. Cases 1 and 6 also resembled the 2 fatal cases of "mucosal respiratory syndrome" reported by Stanyon and Warner,[2f] both clinically and pathologically, but in the present cases the cutaneous lesions were more severe and extensive. The significance of the mucocutaneous lesions and their relation to the pneumonia remain obscure. The case with mitral stenosis resembles the one described by Longcope.[2a]

5. Sturdee, R. L., and Scott, W. M.: A Disease of Parrots Communicable to Man (Psittacosis), Ministry of Health, Reports on Public Health and Medical Subjects no. 61, London, His Majesty's Stationery Office, 1930. Lillie, R. D.: The Pathology of Psittacosis in Man, National Institute of Health Bulletin 161, United States Treasury Department, Public Health Service, 1933. Appelbaum, E., and Ackermann, W.: Ann. Int. Med. 17:528, 1942. Tanner, F. H.; Covey, G. W.; Everett, H. H., Jr.; Everett, H. H., Sr., and Neely, O. A.: Nebraska M. J. 30: 386, 1945.

As is true of influenza, secondary bacterial invasion seems to be the rule, but there is a sharp contrast between the changes produced by such bacterial invasion in influenza and those produced in atypical pneumonia. In influenza secondary staphylococcic invasion causes a fatal disease, with death occurring either in a few days or after two weeks or more.[6] In the rapidly fatal cases the most prominent features are edema and hemorrhage, and extensive necrotizing tracheobronchitis and multiple pulmonary abscesses are also seen. No lesions of such extent occurred in our cases of atypical pneumonia. In cases of influenza of longer duration the lungs show extensive fibrosis and cavity formation. In our series 3 patients with atypical pneumonia had evidence of secondary Staph. aureus infection. All 3 lived three weeks or more, but at autopsy no changes resembling those described in cases of influenza were found. In these cases, therefore, the staphylococcic infection was either terminal or mild.

In 4 of our cases streptococci with alpha hemolysis were cultured from the lungs. While the bronchioles contained numerous polymorphonuclear leukocytes and some cocci in places, there was no pyogenic type of reaction in the bronchial walls or in the alveoli. These streptococci, therefore, probably had no relation to the pneumonic process.

The pathologic picture of atypical pneumonia bears a resemblance to that of the experimental pneumonia produced by McCordock and Muckenfuss[7] in rabbits, utilizing vaccine virus. These authors found that a dilute suspension of virus given intratracheally produced a proliferative cellular lesion, which they termed interstitial virus pneumonia.

All attempts to demonstrate inclusion bodies of any type or the L. C. L. bodies[8] of psittacosis in our material, utilizing various staining methods, yielded entirely negative results. Attempts to isolate a virus were made in 6 cases and were all unsuccessful.

GENERAL SUMMARY

The clinical and pathologic observations of 8 cases of primary atypical ("viral") pneumonia have been presented.

Clinically these cases were characterized by increasing symptoms of respiratory embarrassment, diffuse moist rales and transient areas of atelectasis but no definite signs of consolidation of the lungs. Roentgenographically, there was an extensive miliary soft nodular type of density in the lungs. The serum contained cold agglutinins.

6. Wollenman, O. J., Jr., and Finland, M.: Am. J. Path. **19**:23, 1943. Parker, F., Jr.; Jolliffe, L. S.; Barnes, M. W., and Finland, M.: ibid. **19**:23, 1943.

7. McCordock, R. S., and Muckenfuss, R. S.: Am. J. Path. **9**:221, 1933. Muckenfuss, R. S.; McCordock, H. A., and Harter, J. W.: ibid. **8**:63, 1932.

8. Meyer, K. F., and Eddie, B.: Proc. Soc. Exper. Biol. & Med. **30**:484, 1933.

Two of the cases were complicated by acute hemolytic anemia, 2 others by severe erythema multiforme exudativum and 1 by rheumatic heart disease with mitral stenosis.

The characteristic pathologic changes noted in the lungs grossly were: the congested appearance of the cut surfaces, which were studded with small grayish or dark nodules, and. the hyperemic appearance of the mucosa of the trachea and the bronchi. Histologically, there were a mononuclear type of alveolar exudate, an interstitial infiltration predominantly of plasma cells, and swelling and proliferation of the alveolar lining cells. While the bronchioles not infrequently contained polymorphonuclear leukocytes and occasionally some bacteria, their walls were infiltrated by mononuclear cells, and the epithelium was intact. Bacterial infection played a minor role except in 2 cases, in which there was some abscess formation.

Attempts to demonstrate intracellular inclusions or to isolate a virus were unsuccessful.

INTRA-ARTICULAR CHANGES INDUCED IN RABBITS BY INJECTION OF· TYPHOID SOMATIC ANTIGEN

HERBERT R. MORGAN, M.D.*
BOSTON
AND
GRANVILLE A. BENNETT, M.D.
CHICAGO

PREVIOUS studies have demonstrated that pathologic changes of certain tissues of rabbits follow the administration of a purified somatic antigen prepared from Salmonella typhosa when this is injected intradermally or intravenously [1a] or when it is used for the induction of the Shwartzman reaction.[2] The present observations concern the effects which this same antigen produces on the articular tissues of the rabbit when it is injected into the joint space or when it is employed as an agent for the production of the Shwartzman reaction in the articular tissues.

MATERIALS AND METHODS

The toxic somatic antigen, prepared from cultures of S. typhosa grown in synthetic liquid medium by a technic involving repeated precipitation with alcohol,[1b] was an aliquot of the material employed previously.[1]

Methods of Administration.—(a) Joints: The knee joints of rabbits were shaved and prepared with iodine and alcohol. Antigen, in doses ranging from 0.25 to 0.5 mg., diluted in saline solution, or saline solution alone as a control was injected into the joint space beneath the patella. The total quantity of fluid injected at any one time did not exceed 0.5 cc. Additional injections were made at intervals of from three to seven days for varying periods.

(b) Shwartzman Reaction: With rabbits used for the Shwartzman reaction, 1.0 mg. of antigen was injected into a joint space, and 0.5 mg. intradermally into the shaven skin of a flank. Eighteen to twenty-four hours later, these animals received an intravenous injection of 6 to 10 mg. of antigen in 10 cc. of saline solution.

1. Morgan, H. R.: (a) Am. J. Path. **19**:135, 1945; (b) J. Immunol. **41**:161, 1941.

2. Shwartzman, G.: Proc. Soc. Exper. Biol. & Med. **25**:560, 1928. Morgan.[1b]

Treatment of Tissues.—All specimens except the joint tissues were fixed in Zenker's solution, embedded in paraffin and stained with hematoxylin and eosin. The joint tissues were fixed in formaldehyde solution, decalcified in 5 per cent aqueous nitric acid solution, embedded in celloidin (a concentrated preparation of pyroxylin) and stained with hematoxylin and eosin.

PATHOLOGIC CHANGES INDUCED BY ANTIGEN INJECTED INTO THE JOINT SPACE

The antigen was administered in 0.25 or 0.5 cc. of saline solution (table 1). With several rabbits the same quantity of sterile saline solution was similarly injected into the control joint. Two of the rabbits (119 and 113) had received intravenous injections of somatic antigen (a total of 6.5 mg.) sixty days previously. One (118) had

TABLE 1.—*Data on Experiment in Which Purified Somatic Antigen of Salmonella Typhosa Was Injected Repeatedly into the Knee Joint of the Rabbit*

Rabbit	Total Amount of Antigen Injected, Mg.	Injections	Interval Between Injections, Days	Time of Death After Last Injection	Manner of Death
2	1.5	4	3-4	14 days	Killed with intravenous injection of air
119	1.25	3	3-7	14 days	Killed with intravenous injection of air
49	1.5	4	3-4	28 days	Killed with intravenous injection of air
113	1.25	3	3-7	28 days	Killed with intravenous injection of air
118	1.25	3	3-7	28 days	Killed with intravenous injection of air

received a total of 10 cc. of typhoid vaccine sixty days before the first intra-articular injection of somatic antigen.

Gross Examination.—Twenty-four hours after the first injection of the antigen, the joint was slightly swollen, and the rabbit protected the involved articulation against motion. After seventy-two hours, the joint was moderately swollen, and an effusion was present. These findings increased after repeated injections of antigen and were marked at the time of autopsy, when some of the joints were moderately well fixed in a position of flexion. The joint spaces contained turbid, sticky fluid, and the synovial tissues were hypertrophied and markedly congested. In the animals killed after the longer intervals of time, there was marked atrophy of the periarticular tissues. The control joints appeared normal on gross inspection.

Microscopic Examination.—Stained smears of the intra-articular fluids showed fibrin strands and large numbers of leukocytes with a predominance of polymorphonuclears. The articular fluid of 1 rabbit (118) contained opaque rounded masses of fibrin, ranging up to 2 mm. in diameter and resembling "rice bodies."

The changes occurring in the joint tissues were similar in nature in all animals but were most striking in the animals killed last. The synovial tissues showed marked inflammatory changes characterized by (1) hypertrophy of the lining tissues, (2) diffuse and focal infiltration of both the synovial and subsynovial layers with polymorphonuclears, mononuclears and lymphocytes, (3) congestion and edema and (4) fibroblastic proliferation in the subsynovial layers, especially at the perichondrial margins (fig. 1 *A*). The cartilage showed areas of partial and complete necrosis. In such areas, many empty lacunar spaces were seen, and the matrix appeared fibrillated and uneven. The hypertrophy and clustering of cartilage cells suggested regenerative activity (fig. 1 *B*).

There did not appear to be any consistent or significant differences between the changes elicited in the joints of rabbits that had received intravenous injections of typhoid somatic antigen or typhoid vaccine previously and those resulting from intra-articular injections of antigen in normal animals. The control joints either were normal or showed only minimal inflammatory changes.

Sections of the heart, the lung, the liver, the spleen and the kidney showed no significant changes. Any lesions of these tissues which may have occurred in rabbits 119 and 113 following the previous intravenous injections of somatic antigen apparently had disappeared.

PATHOLOGIC CHANGES FOLLOWING THE PRODUCTION OF THE
SHWARTZMAN PHENOMENON IN THE KNEE
JOINT AND SKIN

The animals in which intra-articular Shwartzman reactions were induced were killed at varying intervals of time as noted in table 2.

Gross Examination.—In the animals that died before there was gross evidence of the Shwartzman phenomenon, i. e., less than two hours after the injection, marked congestion of the viscera, especially of the liver, was observed. The area of skin into which the injection had been made twenty-four hours earlier was inflamed and edematous. An increased amount of turbid and frequently blood-stained fluid was found in the articular spaces of the swollen joints. The synovial tissues of these joints were reddened and edematous.

With the appearance of the Shwartzman reaction, the prepared area of skin became dark red because of extensive extravasation of blood. The periarticular swelling increased, and the skin overlying the medial and lateral portions of the joint showed a dark purplish coloration due to the underlying hemorrhage. In animals 4 and 48, killed at eight hours, the articular tissues were edematous and hemorrhagic, and the involved joints contained an excess of turbid fluid. There were hemorrhages in the walls of the large intestine, and the kidneys had a mottled appearance.

In aimals dying after long intervals (five days) the site of the cutaneous Shwartzman reaction had become blackened, shrunken and depressed. The skin over the involved knee joint showed similar changes. The joint spaces contained purulent exudate, and the reddened synovial tissues extended over the margins of the patellar surface of

Fig. 1.—*A*, proliferative and inflammatory changes occurring in the synovial and subsynovial tissues at the perichondrial border of the articular surface of a femur. (Rabbit 49, table 1, killed twenty-eight days after the last intra-articular injection of antigen.)

B shows, in addition to the inflammatory changes of the synovial tissues, the necrosis of articular cartilage that was observed in areas. Clustering of cartilage cells is also apparent. (Rabbit 118, table 1, killed twenty-eight days after the last intra-articular injection of antigen.)

the femur. In rabbit 124, killed at nine days, the knee joint showed even more extensive proliferation of the synovial tissues. At twenty-eight days (rabbit 133) the knee joints were markedly swollen and the adjacent muscles atrophied. The articular spaces contained an excess of turbid fluid. The greatly thickened synovial tissue had overgrown a thinned and atrophied articular cartilage.

Microscopic Examination.—The synovial fluids obtained from the knee joints at various intervals following the induction of the Shwartzman reaction showed numerous polymorphonuclears, mononuclear leukocytes and erythrocytes. In the animals killed after periods of one-half hour to twenty-four hours, the tissues showed a reaction characterized by capillary hemorrhage and thrombosis of the blood vessels.

TABLE 2.—*Data on Experiment in Which the Shwartzman Phenomenon Was Produced in the Knee Joint of the Rabbit with Purified Somatic Antigen of Salmonella Typhosa*

Rabbit	Severity of Shwartzman Reaction		Time of Death After Intravenous Injection	Manner of Death
	Knee Joint	Skin		
125	0	0	½ hr.	Died
127	0	0	½ hr.	Died
130	0	0	½ hr.	Died
132	+	+	2 hr.	Died
4	++	++	8 hr.	Killed with intravenous injection of air
48	+++	+++	8 hr.	Killed with intravenous injection of air
128	+++	++	24 hr.	Died
134	++++	++++	5 days	Died
124	++++	++++	9 days	Killed with intravenous injection of air
123	++	++	18 days	Killed with intravenous injection of air
133	++++	++++	27 days	Killed with intravenous injection of air
126	(Control—no intravenous injection)		21 hr.	Killed with intravenous injection of air

The synovial membranes were edematous and congested with a heavy diffuse leukocytic infiltration in which polymorphonuclear leukocytes predominated (fig. 2 *A*). These changes became more marked with the passage of time (fig. 2 *B*).

As the intervals following the induction of the Shwartzman reaction lengthened, the process within the joint was enhanced by acute and subacute inflammation. The synovial and subsynovial tissues showed focal areas of necrosis on which fibrin was deposited. The upper third or half of the articular cartilage showed loss of cell structure, and the matrix stained poorly. The early proliferative changes of the fibroblasts observed in the synovial and subsynovial tissues at the perichondrial margins and elsewhere were striking. Some blood vessels showed organizing thrombi. In rabbit 133, killed twenty-seven days after the production of the Shwartzman reaction, there was deformity of the articulation, caused by degeneration of the articular cartilage, inflammation, and fibrosis of the capsular tissues, and productive growth of

Fig. 2.—*A*, congestion of blood vessels with early extravasation of erythrocytes, which have penetrated into the synovial and subsynovial tissues, and pronounced early leukocytic infiltration of the tissues and the joint space. (Rabbit 130, table 2, which died thirty minutes after the intravenous injection of antigen.)

B, congestion, widespread hemorrhage and thrombosis of blood vessels of the synovial and subsynovial tissues. (Rabbit 48, table 2, killed eight hours after the intravenous injection of antigen. A typical Shwartzman reaction was apparent in the knees and the skin.)

cartilage at the perichondrial margin (fig. 3 B). The synovial tissues showed acute and chronic inflammatory changes (fig. 3 A) with extensive leukocytic and lymphocytic infiltration. These changes indicated pronounced and continuing injury of all articular tissues.
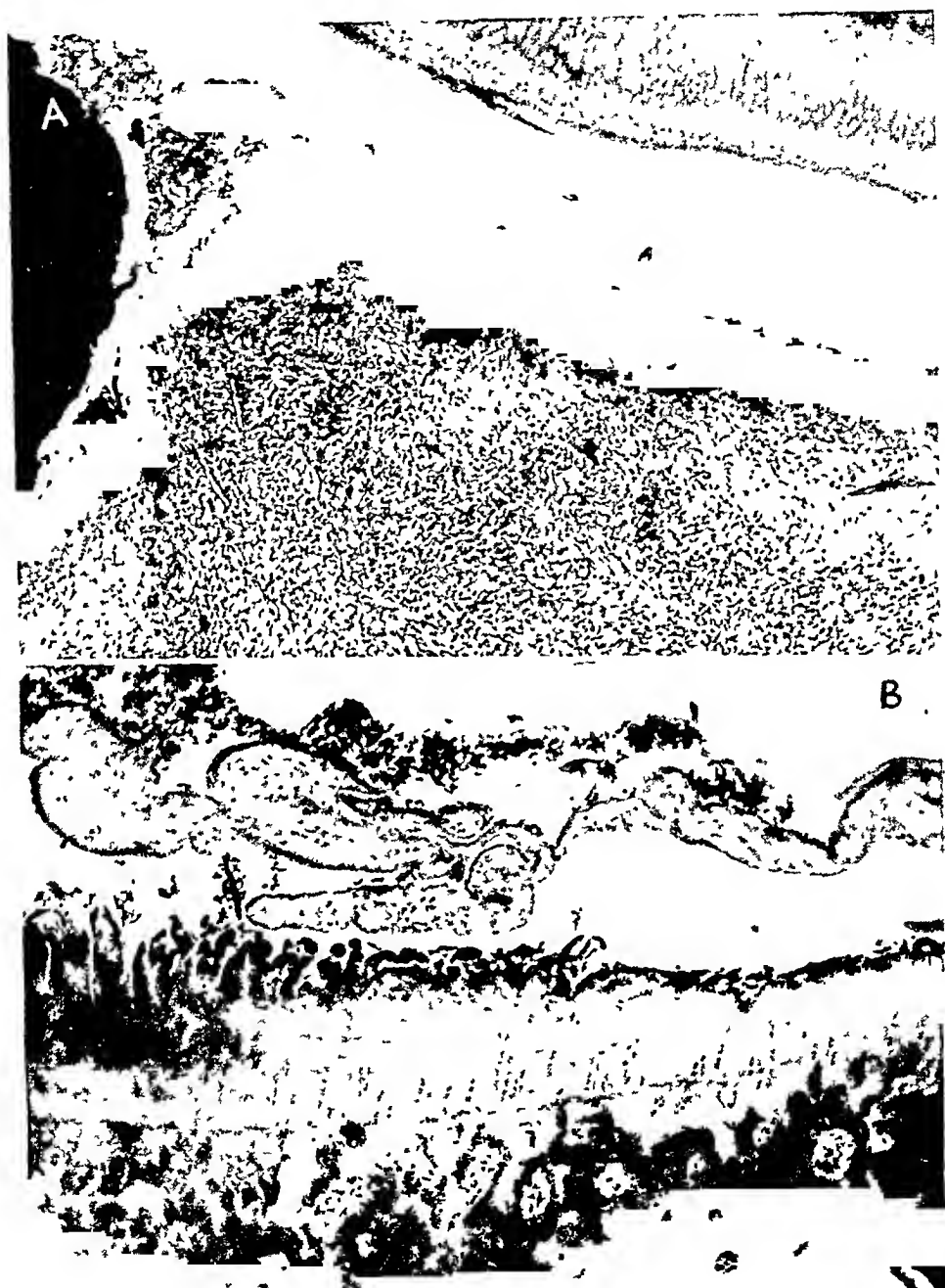
Fig. 3.—*A* shows the synovial and subsynovial tissues extremely thickened and granulation tissue replacing the surface exudate and necrotic tissue twenty-seven days after the production of the Shwartzman reaction. (Rabbit 133, table 2).

B, marked degeneration of the articular cartilage of a femur and a tibia. (Rabbit 133, table 2, killed twenty-seven days after the production of the Shwartzman reaction.)

The area of skin in which the Shwartzman reaction was produced was characterized soon after the onset of the reaction by acute inflammatory changes, hemorrhage, and occasional thrombosis of blood and lymphatic vessels (fig. 4 *A* and *B*). By the end of five days the cutaneous lesion had taken on the character of an eschar with old hemorrhage, necrotic collagen fibers and masses of leukocytes. Later this eschar became sharply demarcated and began to separate from the adjacent
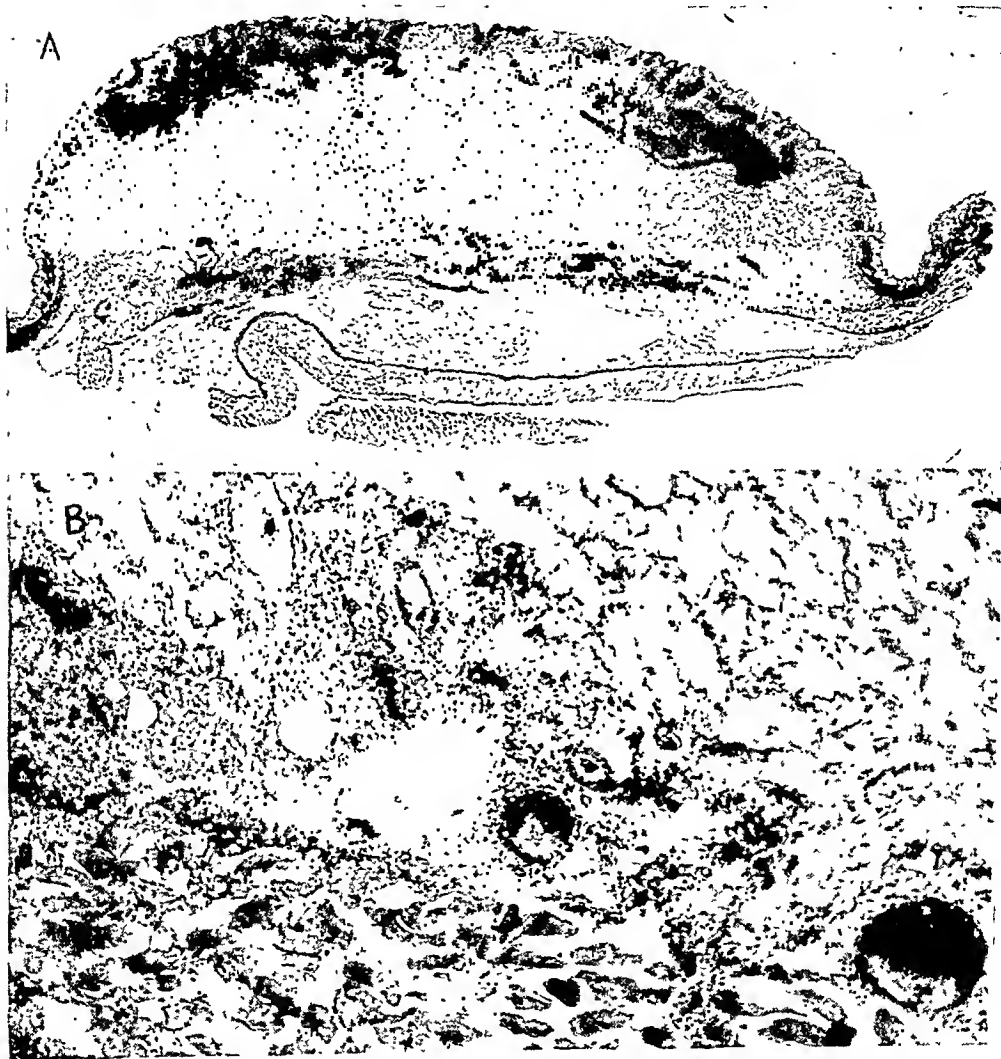
Fig. 4.—*A*, low power photomicrograph showing marked congestion and edema and early hemorrhage in the prepared area of skin eight hours after the intravenous injection of antigen. (Rabbit 48, table 2.)

B, higher magnification of an area of the lesion shown in *A*, revealing edema, leukocytic infiltration, congestion and early thrombosis of vessels.

viable tissues through the formation of granulation tissue (fig. 5). By the eighteenth day this area had either sloughed off or become a sharply circumscribed abscess with a thick fibrotic wall.

The changes observed in the cardiac, pulmonary, hepatic, renal, adrenal and splenic tissues of these animals were similar to those observed following repeated intravenous injections of antigen [1a] with certain exceptions. The heart muscle frequently showed more extensive areas of necrosis. These changes were more marked in the right ventricle, which showed extensive calcification in some animals killed after

Fig. 5.—A line of separation is seen between the necrotic dermal tissues on the right and the viable tissues on the left. The area of the Shwartzman reaction consists of a necrotic plaque or eschar. (Rabbit 124, table 2, killed nine days after the onset of the reaction.)

intervals of more than a week (fig. 6 A). The lungs showed the areas of hemorrhage and the thrombi in blood vessels (fig. 6 B and C) which were noted in the earlier experiments,[1a] but the changes in the blood vessels were more outstanding.

Fig. 6.—*A*, degeneration, fibrosis and early calcification of the myocardium of the right ventricular wall. Near the top of the reproduction are bundles of swollen and vacuolated but viable muscle fibers. (Rabbit 124, table 2, killed nine days after intravenous injection of antigen.)

B, an area of old hemorrhage and fibrosis in the lung. This lesion appeared to have been caused by an injury of blood vessels resulting in thrombosis.

C, higher magnification of an area of lung similar to that shown in *A*. The wall of the pulmonary artery is necrotic and partially calcified. The lumen is occluded by an organizing thrombus. (Rabbit 124, table 2.)

618

In rabbit 126, which was killed twenty hours after it had received an injection of 1 mg. of somatic antigen into each knee joint, only the adrenal among other body tissues showed changes. There was an infiltration with polymorphonuclear leukocytes, which in some instances had invaded the cortical cells. This reaction was similar to that seen in the animals which had received the intravenous, eliciting injection of antigen. The cardiac, pulmonary, hepatic and renal tissues of this animal showed no significant changes. The fact that lesions were observed only in the adrenal tissues suggests that only small amounts of the somatic antigen injected into the knee joints may have gained access to the blood stream.

COMMENT

The results described indicate that the toxic somatic antigen derived from S. typhosa produced marked changes in the articular tissues when injected directly into the joint space. The intravenous injection of typhoid vaccine or somatic antigen made some days previous to the intra-articular injection of somatic antigen had no effect on the type of changes observed in the tissues of the joint. This suggests that the presence of circulating typhoid antibody does not modify the reaction of joint tissues damaged by this toxic antigen. This effect is similar in nature to the destructive action of the antigen on cardiac, hepatic and adrenal tissue and on bone marrow following its intravenous administration.[1a] The latter tissues showed little or no visible change following intra-articular injection of the antigen, suggesting that the antigen did not pass readily from the joint space into the circulation. This observation is in keeping with the previous demonstration of the ability of the antigen to induce rapid inflammatory fixation *in situ.*[1a] However, the changes occurring in the adrenal gland of rabbit 126 suggested that minute amounts of the somatic antigen may have entered the circulation from the joints.

In previous studies [1a] the intradermal injection of the antigen induced a prompt and severe acute inflammatory reaction. The ensuing lesion soon became encased in a wall of dense connective tissue, and complete healing followed.[1a] In sharp contrast, in the present experiments the intra-articular injection of the antigen elicited an acute inflammatory reaction which subsequently became chronic with resulting hypertrophy of the synovial membrane, degeneration of cartilage and gross deformity of the joint. The intensity and the progression of the articular as compared with the cutaneous reaction would seem to indicate that the articular tissues are more vulnerable to this toxic agent.

When used for inducing the Shwartzman reaction in the skin and the joint tissues, S. typhosa antigen caused, in addition to violent acute inflammation, a pronounced vascular injury that resulted in hemorrhage and thrombosis of blood vessels of the adjacent tissues. These changes

were similar to those observed by Moritz and Morley.[3] Again it was observed that the articular lesion was much more persistent than was the dermal lesion. The latter reaction passed through a typical self-limited course with eschar production and prompt segregation whereas the affected joints showed evidence of continued inflammation and proliferation of granulation tissue for as long as twenty-seven days after the Shwartzman reaction had occurred.

The changes observed in the cardiac and pulmonary tissues of these animals may have been due in part to injury resulting from a generalized Shwartzman reaction, for evidence was noted (rabbit 126) that small amounts of the somatic antigen may have entered the circulation after the preparatory intra-articular injections. This material could have acted as a preparatory dose for the subsequent intravenous injection.

SUMMARY

Typhoid somatic antigen when repeatedly injected into the knee joints of rabbits produces acute arthritis, which gradually becomes chronic, leading to hypertrophy of the synovial tissues, with focal accumulations of lymphoid cells in the subsynovial layers, accompanied by destruction of the joint cartilage. No differences were noted between the lesions produced in normal rabbits and those in rabbits that had received previous intravenous injections of typhoid antigens.

When the Shwartzman reaction is induced in the skin and the knee joint, there is extensive vascular injury, resulting in hemorrhage, thrombosis and tissue necrosis. Such lesions undergo repair—in the skin by formation and separation of an eschar, in the joints by productive inflammation·that may remain active for twenty-seven days or longer. These animals may also have areas of necrosis and thrombosis of blood vessels in the heart, the lungs and the liver.

3. Moritz, A. R., and Morley, J. D.: Proc. Soc. Exper. Biol. & Med. **29**:321, 1931.

FIBROSIS UTERI

R. SHOEMAKER, M.D.
AND
J. E. KAHLER, M.D.
LOS ANGELES

THE CRITERIA for the diagnosis of fibrosis uteri have never been clearly defined from either a clinical or a pathologic standpoint. In no textbook, not even in those devoted solely to gynecologic disorders, is an adequate description of the disease given. As a result, some physicians, both clinicians and pathologists, deny the existence of the entity; others accept it with little interest as offering an explanation in rare or isolated instances of menorrhagia; still others, unfortunately, employ the term in a loose manner to label the condition of a uterus which when removed contains no lesion ordinarily acceptable as affording an explanation for the patient's symptoms. On the other hand, the surgeon who has made a preoperative diagnosis of uterine myoma or adenomyosis may encounter at operation only a symmetric, slightly enlarged uterus which when palpated in situ reveals uniformly and not greatly increased consistency. He hesitates to remove an organ showing so little gross pathologic change and may resort to a suspension operation or a similar ineffectual procedure without benefit to the patient.

The slightly enlarged, firm uterus which gives rise to menorrhagia has aroused interest for fifty years or more. A number of papers were written on the subject in the early 1900's. Since then interest has waned. Further knowledge of the endometrial cycle has shown the cases of so-called endometritis to be merely instances of normal secretory activity of the endometrium, but the changes in the myometrium and their possible causation of menorrhagia are still poorly understood.

Scanzoni,[1] in 1863, described these changes and considered them a result of chronic metritis, a conception supported by Bell,[2] von Lorentz [3] and Goodall.[4] Findlay [5] did not agree as to an inflammatory causation;

From St. Vincent's Hospital.

1. Scanzoni, F. W.: Die chronische Metritis, Vienna, L. W. Seidel u. Sohn, 1863, pp. 1-52.

2. Bell, W. B.: The Principles of Gynaecology, New York, William Wood & Company, 1917, p. 265.

3. von Lorentz: Arch. f. Gynäk. 70:309, 1903.

4. Goodall, J. R.; Altimas, G. T., and Ayre, J. E.: J. Obst. & Gynaec. Brit. Emp. 49:18, 1942.

5. Findlay, P.: Am. J. Obst. 52:71, 1905.

he also discarded the possibility that arteriosclerosis was responsible. He considered the changes to be due to an increase of fibrous connective tissue following prolonged chronic passive congestion.   Berkley and Bonney [6] considered the lesion to be fibrosis without any inflammatory basis, in most cases, Novak [7] expressed the belief that in some cases it results from chronic metritis and that in others it represents chronic subinvolution.   In 1907 Shaw [8] wrote his thesis "The Pathology of Chronic Metritis."   He made Van Gieson-stained sections in a series of cases and measured them carefully.   He concluded that the uterus was enlarged by a proportionate hypertrophy of the fibrous and muscular elements. He also divided his cases into four groups depending on the distribution of the connective tissue.   In group 1 the connective tissue occurs in densely staining strands between muscle bundles.   In group 2 these dense strands also extend between the muscle cells.   In group 3 the connective tissue between the muscle bundles is arranged in a loose meshwork, and in group 4 a similar loose meshwork is found between the muscle cells.   He does not elucidate this classification further.

In 1944 Williams and Kinney [9] studied a group of 10 cases.   They found no fibrosis but instead hypertrophy of the muscle fibers and so proposed the term "myometrial hypertrophy."   All their patients were multiparas, and all but 1 patient, 25 years of age, were between 37 and 51 years of age.

Curtis [10] in a recent paper has proposed the term "diffuse hypertrophy of the uterus" to include all the uteri showing moderate diffuse enlargement regardless of the etiologic factors or the pathologic observations.

The confusion of terminology has continued, and the diagnosis has often been made only when no other explanation of the patient's symptoms was found.

The present study was made in an attempt to determine whether the disease is a clinical entity or not and to define the pathologic criteria for making the diagnosis.

MATERIALS AND METHODS

Fresh uteri received from the surgery division were inspected, measured and opened along the middle of the anterior surface. The width and the consistency of the myometrium and the endometrium were noted. The organ was then halved by continuing the incision, and a block of tissue was removed to represent the full width of the uterus in the center of the posterior wall, an area arbitrarily chosen for

6. Berkley, C., and Bonney, V.: A Guide to Gynecology in General Practice, London, Oxford Medical Publications, 1915, pp. 190-191.

7. Novak, E.: Gynecological and Obstetrical Pathology, Philadelphia, W. B. Saunders Company, 1940, p. 159.

8. Shaw, W. F.: J. Obst. & Gynaec. Brit. Emp. **11**:124, 1907.

9. Williams, J. T., and Kinney, T. D.: Am. J. Obst. & Gynec. **47**:380, 1944.

10. Curtis, A. H.: Am. J. Obst. & Gynec. **50**:748, 1945.

purposes of uniformity. The block was fixed in Zenker's solution, to which glacial acetic acid has been added to a concentration of 5 per cent just before use, and stained with Mallory's aniline blue or Masson's trichrome stain. The organ was then sliced by parallel incisions at 5 mm. intervals to demonstrate any additional lesions.

The four layers of the myometrium were identified and marked off on the slide with india ink and wax pencil. These four layers named from within outward are: the submucous, the vascular, the supravascular and the subserous. The dividing lines between them are indistinct because of the interlacing of the muscle bundles, but for the purposes of this study they were delimited as follows: (*a*) The vascular layer, comprising the central two fifths to one half of the myometrium, was identified by its large vessels as seen with an inverted ocular or 32 mm. objective. Its musculature is relatively compact. (*b*) The submucous layer lies between the vascular layer and the endometrium. It is traversed by vessels which run more or less perpendicular to the endometrium. Its musculature is more compact than that of any other layer. Its width is roughly 25 per cent of the myometrium. (*c*) The supravascular layer and (*d*) the subserous layer lie external to the vascular layer and together represent approximately 25 per cent of the width of the myometrium. The subserous layer is very thin and composed of two longitudinal layers separated by an oblique one but the whole very compactly arranged and thus easily distinguished from the supravascular layer, in which the small muscle bundles are loosely arranged in a bulky supporting connective tissue.

The width of each layer was measured with an ocular micrometer, a 4 mm. objective being used, and the fibrous connective tissue content of each layer was measured in the same manner. In the process of measurement all tissue staining blue with Mallory's stain or green with Masson's was considered fibrous connective tissue, and the artefactural spaces within this tissue were measured as though the fibrous tissue were solid. Since the same method of measurement was used in both the cases of fibrosis and the controls, this error is not significant in a comparison of the two; however, it does magnify the apparent fibrous tissue content of all the uteri studied.

Measurement of individual muscle fibers and counting of muscle fibers in a given area were also done in repetition of the work of Williams and Kinney.[9]

In this manner 27 uteri with fibrosis uteri and 28 control uteri were studied. The control group consisted of autopsy and surgical specimens which were normal or contained leiomyomas, adenomyosis or endometrial polyps and which came from patients whose age distribution was the same as that of the patients with fibrosis.

RESULTS OF STUDY

Incidence.—The 27 cases of fibrosis uteri were found in a period of nine months, during which a total of 245 uteri were received for examination, an incidence of 1 in 9, or 11 per cent.

Twenty-six of the patients were Caucasian; one was Negro.

Sixteen were multiparous and 5 primiparous, and 1 was nulliparous. In 5 cases the parous state was not noted.

The youngest patient was 31 years of age; the oldest, 52. The average age was 41 years. Eleven patients were in the fourth decade, 13 in the fifth and 3 in the sixth.

Menstrual History.—The age of onset of menstruation was not remarkable, nor was any correlation found between the early menstrual history and the later development of fibrosis.

All patients but 2 complained of profuse menorrhagia. The exceptions were, first, a 40 year old nullipara operated on for appendicitis, who had always had regular menstruation at twenty-eight day intervals with moderate flow and without pain. The uterus contained a 7 mm. subserous leiomyoma and was moderately enlarged. The myometrium was found to contain 22 per cent fibrous tissue in the submucous layer. The second exception was a 42 year old multipara sexigravida whose uterus measured 6 by 5 by 3.5 cm. A cervical repair done nine months previously, after the birth of her last child, had been followed by cervical stenosis with severe dysmenorrhea but without menorrhagia. Her uterus contained 25 per cent fibrous tissue in the submucous layer.

The character of the menorrhagia in the remaining 25 cases was described as "heavy," "profuse" or "flooding" and lasted for periods varying from that of the patient's customary duration to twenty days. In 1 case hysterectomy was performed ten days after the onset of severe menorrhagia, but the majority of the patients had had the symptoms for from two to three years, while the longest duration of symptoms was seven years. The periods remained regular and at former intervals in one third of the cases, while in the remainder they became irregular and occurred at shorter intervals.

In only 1 patient did dysmenorrhea develop with the menorrhagia, while in 2 others the menstrual pain usually experienced was increased in severity and became cramplike in character.

None of the patients had metrorrhagia.

In some cases the date of the last delivery antedated the onset of symptoms by twenty years. In only 1 instance was the last delivery within the same year as the onset of symptoms.

Prior Therapy.—In a few of the cases estrogens had been administered without change in symptoms.

Preoperative Clinical Diagnosis.—The clinical diagnosis in these 27 cases were: leiomyoma in 21, adenomyosis in 1, stenosis of the cervix in 1. A preoperative diagnosis of fibrosis uteri was made correctly in the remaining 4 cases.

On pathologic examination one or more additional lesions were found in 15 of the 27 cases. In 11 cases these lesions were subserous or intramural leiomyomas (measuring as much as 7 mm.); in 2, adenomyosis; in 3, small endometrial polyps, and in 2, cervical polyps. With the possible exception of the endometrial polyps, these additional lesions were not considered adequate to explain the patient's severe menorrhagia or the consistency of the myometrium.

Gross Appearance of Uterus.—In 1 case the uterus was normal in size, measuring 5 by 4 by 3 cm. In all the remaining cases it was enlarged.; the average measurements, excluding cervix, being 7.2 by 6.1 by 3.8 cm.; the largest measured 11 by 7.5 by 6 cm. The outline was normal. The myometrium averaged 2.6 cm. in width (extremes, 1.5 to 4 cm.; normal, 1 to 1.3 cm.). The color was pale pink to dull white. The fresh cut surface was either flat or bulged slightly, and, except for the prominent vessels in the vascular layer, was finely textured in contrast to the coarsely trabeculated bulging cut surface of a uterus with adenomyosis. The consistency was increased in all cases but not always uniformly; that is to say, it was increased to a moderate to marked degree in the inner one third to one half of the myometrium in every case and was increased in the outer half in only half the series, being normal beyond the vascular layer in the remainder. The endometrium showed no notable changes.

On the basis of the menstrual history, the hysterectomy was performed within a week of the next menstrual period in 2 cases, in the first week following menstruation in 1, in the second week in 5 and in the third week in 6, while in the remainder it was performed during a period of menorrhagia continuous with or part of the menstrual period.

Microscopic Appearance.—In the normal myometrium the smooth muscle fibers are collected into various-sized bundles which interlace in all directions. Separating the muscle bundles are variable amounts of fibrous tissue, while surrounding each individual muscle fiber is a thin connective tissue sheath. This connective tissue is made up of collagen fibers, elastic fibers and reticulum fibrils. In fibrosis uteri an abnormal amount of connective tissue occurs both between the muscle bundles and, more characteristically, around the individual muscle cells. As well as could be determined, this increase is in collagen and not in elastic fibers or reticulum. Most of the cases fell into Shaw's class 2. This increase of connective tissue is, in some cases, apparent on staining with hematoxylin and eosin, but more often Mallory's or Masson's stain is necessary to demonstrate the abnormality, while occasionally accurate measurement is essential for a diagnosis.

The results of the measuring of the fibrous tissue content of the myometrium are shown in the accompanying table. There is an appreciable increase in the fibrous connective tissue throughout the myometrium in fibrosis uteri as compared with controls; however, there is considerable overlapping of this content in the two series with respect to the figures for myometrium as a whole as well as those for the vascular and supravascular layers. A significant and diagnostic difference, however, occurs in the submucous layer: In all of the cases of fibrosis this layer contained 15 per cent or more fibrous tissue, while in all but 6 of the

controls it contained less than 15 per cent. In 3 of the exceptions it contained 15 per cent, and in 3, 17, 21 and 23 per cent, respectively. Of the 6 women represented in these exceptions, 2 were multiparous, 3 primiparous and 3 nulliparous. One of these patients, aged 52, was nearing the end of the menopause. Her uterus was removed for multiple large tumors, diagnosed as leiomyoma. She did not complain of menorrhagia. Another had hysterectomy for acute endometritis, cause undetermined, in the first trimester of pregnancy. This patient had had mild menorrhagia, preceding the pregnancy, and her condition might conceivably represent an early stage in the development of fibrosis uteri. Three additional women had hysterectomy for leiomyoma and the last for extensive cervical laceration. No factor common to these 6 patients could be found; their ages varied from 21 to 52 years.

That the major localization of the fibrosis is in the submucosal layer is particularly interesting in view of the studies of Faulkner[11] who, while investigating the blood supply of the uterus, found the submucosal

Fibrous Tissue Content of Myometrium

	Submucous Layer *	Vascular Layer *	Supravascular Layer *	Entire Myometrium *
Fibrosis uteri				
Average	21.9	24.9	33.6	27.7
Extremes	15-35	11-33	8-50	19-37
Controls				
Average	11.8	16.7	28.6	20.0
Extremes	3-23	9-27	13-42	11-27

* The value is the percentage of the width.

zone poorly injected during the follicular phase of the cycle but well injected during the luteal phase, as well as in those patients bleeding at the time of surgical treatment. This probably explains the difference in appearance between Shaw's classes 1 and 3, and 2 and 4, since we noted in our own cases that the fibrous connective tissue, whether it is interfascicular or intrafascicular, appeared looser during the luteal phase of the cycle than during the follicular phase. However, in our cases the consistency of the myometrium and the actual amount of fibrous tissue present in it were definitely increased, regardless of the phase of the menstrual cycle during which the uterus was removed.

William and Kinney concluded that the increased consistency of the myometrium in fibrosis uteri is due to hypertrophy of the individual muscle fibers as measured in sections stained with hematoxylin and eosin. We found that the distinction between the muscle cell and its fibrous sheath with this stain was poor, so that measurement of cell diameters

11. Faulkner, R. L.: Am. J. Obst. & Gynec. **49**:1, 1945.

was carried out on sections stained with Mallory's or Masson's stain. In only 3 of the 27 cases of fibrosis uteri was the cell diameter found increased as compared with the controls. In these 3 cases the fibrous tissue content of the submucous layer was 15, 18 and 26 per cent, respectively.

In addition, the muscle fibers present within a space 158 microns wide were counted, and the only cases of fibrosis uteri in which the fibers in this space were significantly fewer as compared with the controls were the 3 instances aforementioned in which muscle fibers were hypertrophied.

In no case were leukocytes found outside the vessels in the myometrium.

Adnexal Lesions.—The tubes and ovaries, whether examined in situ at operation or grossly after being removed with the uterus, revealed no lesion present in a sufficient number of cases to be considered of importance in the etiology of the uterine changes.

SUMMARY

The patient with fibrosis uteri may be described as having a history of profuse menorrhagia without metrorrhagia and usually without dysmenorrhea. Her age may be any age between 30 and 55 years. She may be nulliparous or primiparous but most commonly is multiparous. On pelvic examination the uterus is found symmetrically enlarged and in normal position. The excised uterus is revealed as a slightly to moderately enlarged organ. The myometrium is pale, finely textured, and thickened, with consistency definitely increased throughout or at least in its inner half. On microscopic examination an increase of fibrous tissue is observed in the submucous layer of the myometrium equaling or exceeding 15 per cent of the width of this layer and appearing as an increase of both the interfascicular and the intrafascicular connective tissue.

This study does not reveal the exact causation or pathogenesis of the lesion but does provide ample evidence that it is not an end stage of chronic metritis, that hypertrophy of muscle fibers plays a minor role (occurring in only 11 per cent of the cases) and that it is not a form of subinvolution of the uterus.

CONCLUSION

From the data presented, fibrosis uteri may be considered a fairly well delimited clinical and pathologic entity. The lesion consists of fibrosis of the myometrium, most marked in the submucosal area, a lesion which at the same time separates this entity from simple muscular hypertrophy and subinvolution and establishes a defect of the myometrium as a causative factor in menorrhagia.

• PARATHYROID ADENOMA WITH GENERALIZED METASTATIC CALCIFICATION

MILLINGTON O. YOUNG, M.D.
NEW HAVEN, CONN.
AND
BÉLA HALPERT, M.D.
OKLAHOMA CITY

R ECORDED instances of metastatic calcification associated with an adenoma of one of the parathyroid glands are few. According to Mulligan,[1] there are only 16 recorded cases of metastatic calcification in which necropsy observations are available. Hyperparathyroidism usually produces characteristic, though varied, clinical manifestations, and therefore may be recognized clinically. In this paper, a case is reported in which signs and symptoms suggestive of hyperparathyroidism were present, yet the condition was not identified until the postmortem examination was completed. Because of the diagnostic problem encountered and the rarity of generalized metastatic calcification, this case is presented in some detail.

REPORT OF A CASE

G. F., a white man aged 49, was apparently well until April 1945, when he began to have frequent episodes of vomiting, not related to meals. A few days later, frequency of urination was noted and nocturia, with micturition up to eight times a night. The urine seemed "unusually warm." A dull lumbar ache and vague pains of the arms and legs were noted. The patient continued working until May 13, when he became mentally confused. His physician treated him with penicillin for "bad teeth and pus in the urine."

The symptoms persisted, the mental confusion increased, and on May 23 the patient was sent to a psychiatric institution. After intravenous administration of dextrose in saline solution and Hartmann's solution, his mental state seemed to improve. On May 29 he was transferred to the University of Oklahoma Hospitals. During this admission the highest blood pressure recorded was 146 systolic and 80 diastolic. On one occasion the blood nonprotein nitrogen was 67 mg. per hundred cubic centimeters. Occasional granular casts were noted in the urine, but no albumin was detected. The specific gravity ranged from 1.008 to 1.022. Up to 30 white blood cells and 40 red blood cells per high power field were counted in the urine at various times. He was discharged June 30, with the diagnosis of subsiding acute glomerulonephritis, improved but unable to resume his work as

---

1. Mulligan, R. M.: Arch. Path. **43**:177, 1947.

a carpenter. One month before his readmission, Nov. 6, 1946, a cold and cough developed, soon followed by increased weakness and pain of the arms and legs.

At the time of readmission he was well developed, well nourished and appeared subacutely ill. The sensorium was somewhat clouded. His temperature was 99 F. The retinal vessels were tortuous; there were no hemorrhages. The teeth were carious, and there was marked alveolar pyorrhea. Just above the manubrium there was a firm mass, 2 cm. in diameter, apparently attached to the trachea. The lung fields were clear to auscultation and percussion. A soft systolic murmur was heard over the tricuspid, aortic and pulmonic valve areas. The pulse rate was 76, with the rhythm regular. The blood pressure was 168 systolic and 90 diastolic. The remainder of the physical examination yielded no pertinent information.

The urine was acid, with a specific gravity of 1.008, a trace of albumin and no glucose; microscopic examination revealed a few red blood cells and epithelial cells per high power field and no white blood cells or casts. The red blood cell count was 5,260,000; the hemoglobin content was 14.5 Gm.; the white blood cell count was 12,750, with neutrophils 71, lymphocytes 26 and monocytes 3 per cent. The plasma proteins were 6.9 Gm. per hundred cubic centimeters, with an albumin-globulin ratio of 5.1 : 1.8. The blood nonprotein nitrogen was 102 mg. per hundred cubic centimeters. Examination of the spinal fluid gave negative results. Roentgenograms of the chest revealed no significant changes.

Symptomatic medications and intravenous injections of fluids were administered. Frequent injections of a morphine salt were required for relief of the chief complaint, pain of the arms and legs. The blood nonprotein nitrogen fluctuated between 35 and 150 mg. per hundred cubic centimeters. The patient's condition became progressively worse, and on November 18 the restlessness and disorientation were marked. Rales were heard throughout both lung fields. The pulse was feeble; the rate, 130. The blood pressure was 100 systolic and 70 diastolic. The red blood cell count was 3,660,000; the hemoglobin content, 11 Gm. The plasma proteins were 5.8 Gm. per hundred cubic centimeters, with an albumin-globulin ratio of 3 : 2.8. The patient vomited frequently for several hours on the day of his death, November 20. The clinical impression was chronic glomerulonephritis with terminal uremia.

Necropsy (seven hours after death).—The body was 167 cm. long and weighed approximately 150 pounds (68 Kg.). A thin brown liquid oozed from the mouth. The abdomen was slightly distended. No other changes were noted on external examination.

The peritoneal cavity contained no excess fluid. The stomach was distended with air and 500 cc. of a thin brown liquid. The entire duodenum and the proximal 16 cm. of jejunum were distended up to 5 cm. in diameter and contained a thin brown liquid. Distal to this portion of the jejunum, a segment 12 cm. long was thin, friable, discolored red-gray-black and covered with fibrin. The branch of the superior mesenteric artery supplying this segment was firm and filled with a thrombus. Distal to the infarcted portion the distention lessened gradually and disappeared in the ileum.

The pleural and pericardial cavities contained no excess fluid; their surfaces were smooth and glistening. The heart measured 8.5 cm. from base to apex and 9 cm. across the base; it weighed 375 Gm. There were no valvular lesions. The trachea and bronchi were filled with a thin brown liquid similar to that in the stomach and the oral cavity. The posterior portions of the lungs were lumpy; the cut surfaces were mottled red and gray. The left lung weighed 775 Gm. and the right 1,000 Gm.

The spleen weighed 170 Gm. and the liver 1,900 Gm.; they were otherwise unremarkable. Cholesterosis with some polyp-like projections was noted in the gallbladder.

The left kidney measured 13 by 7 by 5 cm. and weighed 225 Gm. The cut surfaces did not bulge and were pale. The cortical and medullary markings were indistinct. The capsule stripped with slight difficulty, leaving a roughened, gray-pink surface. The mucosa of the calices, pelvis and ureter was pale and delicate. There were no calculi. The right kidney resembled the left and weighed 210 Gm. The urinary bladder contained some cloudy yellow urine and no concretions. Its wall was of usual thickness, and the mucosa was slightly injected.

In the isthmus of the thyroid gland there was a partly calcified nodule, 2 cm. in diameter; two softer nodules were contained in the lower pole of the right lobe. On the anterior surface of the body of the first thoracic vertebra, immediately to the left of the esophagus, there was an encapsulated pink-yellow soft mass. It was not connected with the thyroid gland, the thymus, or other nearby structures. The mass was fusiform, 6 by 2.5 by 1.5 cm., and weighed 28 Gm. The cut surfaces were mottled pink and yellow; they showed cavities measuring as much as 0.4 cm. in diameter, containing clear fluid (fig. 1).

The pancreas, the adrenal glands and the genital organs were not remarkable. No gross abnormalities of the skeleton were apparent. Examination of the cranial contents was not authorized.

Microscopic preparations stained with hematoxylin and eosin revealed that the mass from the left posterior part of the superior mediastinum was a para-thyroid adenoma (fig. 2). Broad sheets of cells having large compact or vesicular round nuclei with a halo of cytoplasm or with indistinct cytoplasmic borders (chief cells) were seen in a scanty connective tissue stroma containing the blood vessels. There were scattered occasional cells with a pink-stained cytoplasm (oxyphil cells). No acinous structures were seen. Within the substance of the mass there were some empty spaces bordered by compressed cells or a delicate membrane-like line. In the scanty connective tissue stroma there were streaks of deposits stained lavender or blue. The lumens of occasional blood vessels were obliterated and in part recanalized (fig. 3).

In the heart, focal areas of lavender-stained deposits were seen in muscle fibers, in the connective tissue and in the walls of blood vessels (fig. 4). In the lungs, similar deposits were seen in some of the septums and in blood vessels (fig. 5). The calcific changes were marked in the capsule, the septums and the blood vessels of the spleen.

In the kidneys the renal pattern was partly obliterated, with some glomeruli hyalinized or replaced by lavender-stained granular deposits. Some glomeruli had thickened Bowman's capsules (fig. 6). The tubules surrounding the hyalinized glomeruli were few, and the stroma contained lavender-stained deposits and an infiltrate of lymphocytes, plasma cells and large mononuclear cells. Elsewhere, occasional glomeruli appeared intact, and the tubules surrounding them were distended. Only occasional blood vessels were intact. Many contained calcific deposits in their walls and thrombi in their lumens.

The stroma and the blood vessels of the stomach, the adrenal glands and the lymph nodes also contained focal calcific deposits.

In the three adenomas of the thyroid gland, particularly in the one located in the isthmus, deposits stained lavender or blue seemed to replace acini or groups of acini. They were also seen in the ground substance of the septums and within the walls of blood vessels.
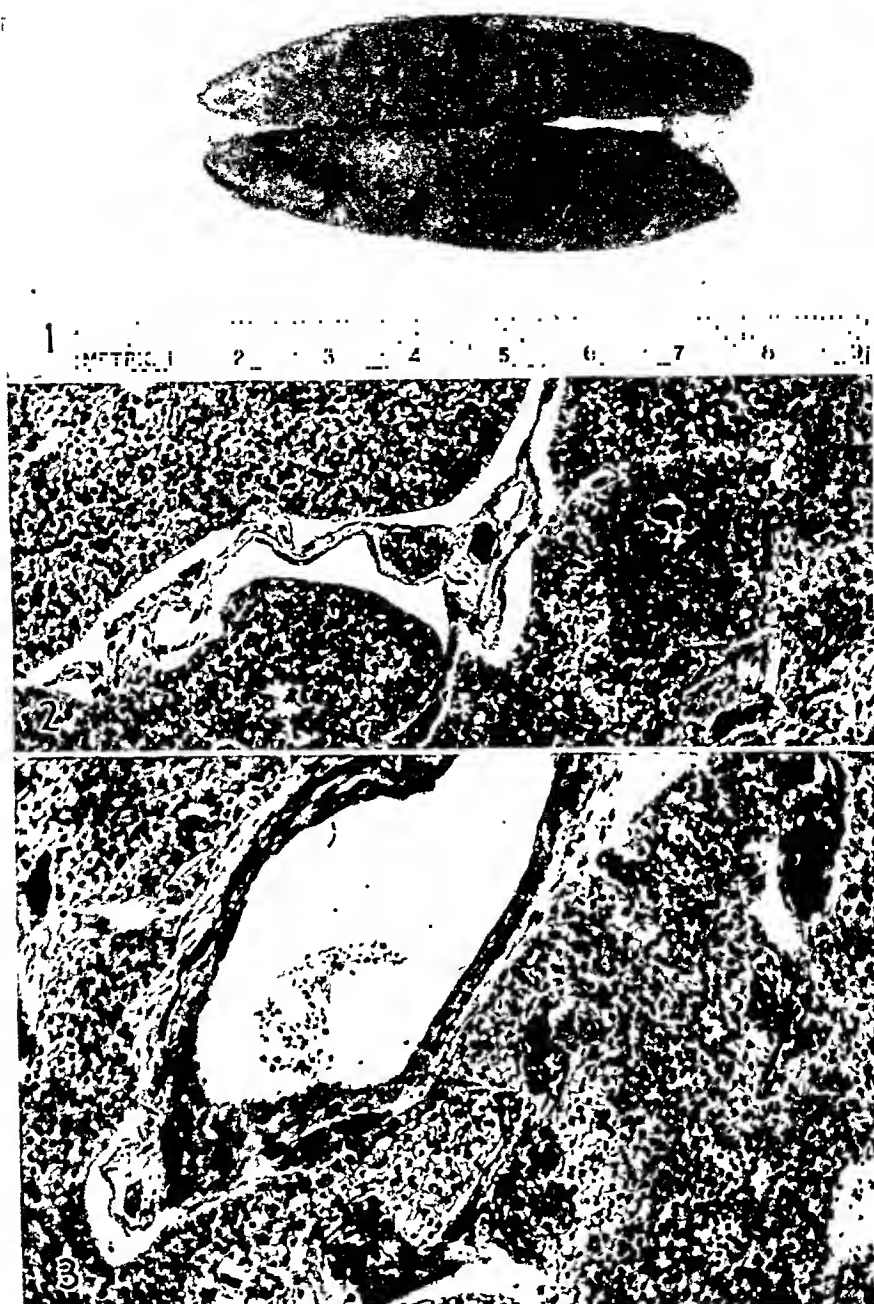
Fig. 1.—Cut surfaces of the adenoma of the parathyroid gland located in the left posterior part of the superior mediastinum. It measured 6 by 2.5 by 1.5 cm. and weighed 28 Gm.

Fig. 2.—Microscopic appearance of the parathyroid adenoma; × 125. Broad sheets of cells having large compact or vesicular nuclei in a halo of cytoplasm are seen in a scanty connective tissue stroma.

Fig. 3.—Parathyroid adenoma; × 125. In the scanty connective tissue stroma there are streaks of calcific deposits stained lavender or blue. The lumens of occasional blood vessels are obliterated and in part recanalized.

Fig. 4.—Metastatic calcification of the myocardium; × 125. Areas of lavender-stained calcific deposits are seen in muscle fibers, in the connective tissue and in the walls of blood vessels.

Fig. 5.—Metastatic calcification of the lungs; × 125. In the septums and the blood vessels of the lung there are calcific deposits similar to those in the myocardium.

Fig. 6.—Metastatic calcification of the kidneys; × 125. The renal pattern is partly obliterated, with some glomeruli hyalinized or replaced by lavender-stained granular deposits.

The calcific deposits were present in the aorta and in arteries within all the viscera examined with the exception of the liver. Lavender-stained deposits were seen in the intima and the inner portion of the media. The lumen of the artery supplying the infarcted segment of the jejunum contained a thrombus. Irregular streaks of lavender-stained granular deposits were seen inside and outside the frequently interrupted inner elastic lamina of the media. Neutrophilic granulocytes had infiltrated the media, the adventitia and the surrounding adipose tissue, which was spread apart.

COMMENT

According to Norris,[2] 322 cases of adenoma of the parathyroid glands were reported between 1903 and 1945. One of the inferior pair of glands was the site of the adenoma in 83.8 per cent of the cases. The adenoma was in an aberrant location in 10.7 per cent of the cases, and the location was in the mediastinum in 63.3 per cent of this group. Thus, a parathyroid adenoma located in the mediastinum, as in the case herein reported, is uncommon, observed in less than 7 per cent of the recorded cases. Adenoma of the parathyroid glands is rarely palpable. When located in the mediastinum it is even less accessible. The observation that the inferior pair of parathyroid glands may be aberrant in the neck and the mediastinum is explained by the fact that they are in close relationship with the primordia of the thymus at the time at which they are derived from the third pharyngeal pouch and are descending in the neck during embryonic life. Because of this close relationship, the inferior pair of parathyroid glands was termed by Weller[3] the "parathymus glands." In addition to the developmental factor responsible for the aberrant locations, Cope[4] suggested a contributing factor. He believed that an enlarging adenoma meets less resistance in growing downward and thus may come to lie in either the anterior or the posterior part of the superior mediastinum. Such a growth usually remains connected to its site of origin by an elongated vascular pedicle.

According to Mulligan,[1] the four underlying causes of metastatic calcification are, in order of frequency, skeletal disease, chronic renal disease, neoplasms of the parathyroid glands and hypervitaminosis D. The organs most frequently involved in the calcific process are the kidneys, the lungs, the heart, the systemic arteries and less frequently the stomach and other organs. Mulligan reviewed the factors involved in the deposition of the calcium found in the soft tissues. When this is due to the activity of a parathyroid adenoma, the chief factor responsible is apparently the state of the blood, which is supersaturated with calcium withdrawn from the skeleton. Sites favorable to such deposition are

2. Norris, E. H.: Internat. Abstr. Surg. **84**:1, 1947.

3. Weller, G. L. Jr.: Contrib. Embryol. **24**:95, 1933; Publication 443, Carnegie Institution of Washington, 1933.

4. Cope, O.: Ann. Surg. **114**:706, 1941.

those with a low hydrogen ion concentration. Organs supplied with blood of low carbon dioxide or high oxygen content (lungs, heart, systemic arteries) and organs which excrete acids (kidneys, stomach, lungs) have a relatively low hydrogen ion concentration. The tissue injury due to the initial calcific change and the degree of phosphatase activity are additional factors to be considered.

The average duration of the symptoms of hyperparathyroidism due to an adenoma is probably five to seven years, according to Norris,[2] who stated that few cases had been diagnosed with symptoms present less than two years. In our case the patient was well until nineteen months before his death.

In retrospect, the patient had symptoms typical of hyperparathyroidism, such as weakness, pains of the back and extremities, and also urinary complaints. The latter, however, were not the kind usually produced by renal calculi. The mental confusion may have been caused by the hypercalcemia or by the multiple thrombosis of small cerebral vessels.

The hypercalcemia, undoubtedly present, though its degree was not determined, must have had its source in the skeleton. Review of the only roentgenograms taken, those of the chest, disclosed no osteoporosis. At necropsy no obvious changes were noted in the skeleton.

Microscopic deposits of calcium were observed in the kidneys, heart, lungs, stomach, spleen, adrenal glands, lymph nodes and their blood vessels. They were also observed in the adenomas of the thyroid gland and within the stroma of the parathyroid adenoma. The calcium deposited in glomeruli, tubules, stroma and blood vessels must have seriously interfered with renal function. The calcium deposited in a branch of the superior mesenteric artery caused formation of a thrombus. This in turn caused infarction of the jejunum with early peritonitis, distention of the proximal jejunum, the duodenum and the stomach, and vomiting. Aspiration of the vomitus aggravated the pneumonia, which was the immediate cause of death.

SUMMARY

Extensive metastatic calcification occurring in a white man aged 49 with hyperparathyroidism is reported. It was caused by an adenoma of the left inferior parathyroid gland, which was located in the posterior part of the superior mediastinum. The condition was not diagnosed during life. Death was due to the sequelae of thrombosis of a branch of the superior mesenteric artery caused by calcific change in its wall.

RETICULOENDOTHELIAL CELLS REACTING TO TOXIC ANTIGENS AND TO INFECTION

P. BUENO, V.M.D.

SÃO PAULO, BRAZIL

IN A PREVIOUS PAPER [1] I have shown that normal horse serum intraperitoneally injected into guinea pigs sensitized to this antigen causes lesions in certain elements of the reticuloendothelial system. This phenomenon was observed in the Kupffer cells of the liver and in the germinal cells of the lymph node cortex and of the lymph follicles of the spleen.

In further studies changes were found in these same elements after the injection of bacterial extracts. This observation led me to make systematic investigations in this direction. Thus the results reported here refer to studies of lesions observed in reticuloendothelial cells in various organs after injection of toxic antigens or during an infectious process.

MATERIAL

Guinea pigs weighing between 250 and 450 Gm. were used, and the following antigens were injected: extract of Salmonella paratyphi A (a filtrate of disintegrated bacteria in isotonic solution of sodium chloride), brucellergin (prepared according to the Huddleson technic), tetanus toxin (200,000 minimal lethal doses [mice] per cubic centimeter), diphtheria toxin (1/1500 minimal lethal dose) and living Bacillus anthracis. Most of the injections were subcutaneous, but the first two antigens were also introduced by the intracardiac route.

The 49 guinea pigs used in this experiment were divided into groups as follows: 11 guinea pigs were given brucellergin in doses of 1 and 2 cc.; 8 received salmonella extract in doses of 1 to 5 cc.; 10 were given tetanus toxin in doses of 1 cc. of a 0.4 : 1,000 dilution and 0.5 and 1 cc. of undiluted toxin; 8 received 0.1 and 0.2 cc. of diphtheria toxin, and 12 animals were infected with 0.5 cc. of B. anthracis suspension in isotonic solution of sodium chloride.

The animals which received the first four antigens were either killed or died, most of them within seven, twenty-four and thirty hours after the injection. The deaths of those infected with B. anthracis occurred from two to four days after the inoculation.

A large series of normal guinea pigs and one of guinea pigs given injections of nontoxic antigens were studied as controls. For the latter such antigens as egg albumin and normal horse serum were used.

From the Department of Pathology, Instituto Biológico.

1. Bueno, P.: Arch. Path. **42**:412, 1946.

From the animals given injections the following material was taken and studied histologically: the lymph nodes, the spleen, the liver and the bone marrow. The material was fixed in solution of formaldehyde U.S.P. diluted 1:5, embedded in paraffin and sectioned and the sections were stained with hematoxylin-eosin.

HISTOLOGIC OBSERVATIONS

Liver.—In most cases histologic study revealed that heavy damage had occurred in a great number of the Kupffer cells.
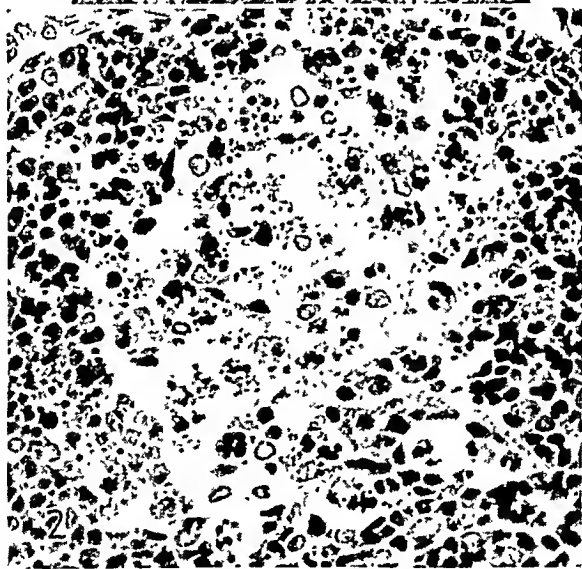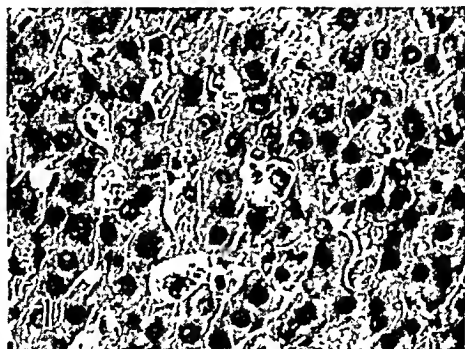
Fig. 1.—Liver after a subcutaneous injection of diphtheria toxin. Note the disintegrating Kupffer cells. × 350.

Fig. 2.—Lymph follicle of spleen showing regressive alterations. Note nuclear pyknosis and cell particles. × 420.

It was observed that the initial cell change is a marked nuclear tumefaction; at first the nucleus is enlarged and slightly stained; stain affinity is increased later, and the beginning of necrobiosis is observed. Then successive phases of contraction, hyperchromatosis and finally nuclear disintegration can be noted (fig. 1). More rarely disintegration of nuclei is seen during the state of tumefaction. In some cases these cells present evident decrease in number, and only cell particles can be seen in their place.

Spleen.—The spleen was also observed to be a site of lesions. These occurred, however, only in the lymph follicles, where evident necrobiotic changes were found (fig. 2). Cells with enlarged or pyknotic nuclei, as well as cell particles, either dispersed or within phagocytes, were noted. It seemed that only germinal cells or slightly differentiated cells presented lesions. The more developed elements did not show changes.
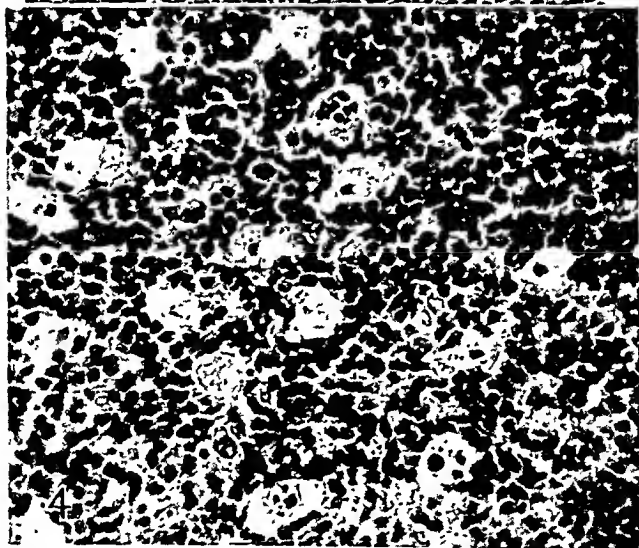
Fig. 3.—Lymph follicle of a lymph node after B. anthracis infection. It shows germinal cells in a state of necrobiosis. × 200.

Fig. 4.—Lymph node after a subcutaneous injection of diphtheria toxin. Extrafollicular germinal cells are seen in a state of necrobiosis. × 400.

No lesions were observed in the reticuloendothelial cells of the pulp. In these cells only nuclear tumefaction was noted.

Lymph Nodes.—The lymph nodes had cell lesions, but these seemed to be confined to the follicular and extrafollicular germinal cells of the cortex.

Generally, marked structural changes appeared within the germinal centers, where the undifferentiated cells or the elements that were in an early stage of differentiation showed distinct necrobiotic changes (fig. 3). Cells with nuclear tumefaction or pyknosis could be seen besides other elements that were already disintegrated. Marked cellular rarefaction was therefore evident, which in more advanced phases implicated the whole follicle. Sometimes almost all the cells completely disappeared.

The extrafollicular germinal cells which are scattered throughout the cortex showed similar lesions. The nuclei of some were enlarged, and the cytoplasm contained cell particles or the cells presented necrobiosis, in sharp contrast with the surrounding normal tissue (fig. 4).

No significant changes were found in other reticuloendothelial elements.

Bone Marrow.—No lesions were observed in cells of this tissue.

COMMENT AND CONCLUSIONS

The present observations show that certain cells of the reticuloendothelial system are damaged when toxic antigens are introduced into the organism or during infection. Particularly interesting, however, is the fact that was emphasized initially, that these are the same cells which react during the anaphylactic phenomenon. This shows that certain undifferentiated mesenchymal cells have a special capacity of reacting to different kinds of antigenic stimuli.

On the other hand, the results confirm a series of researches which indicated that toxins damage lymphatic tissue. My observations showed, moreover, that lesions occur not only in the germinal elements of the lymphatic tissue but also in cells which are considered as a source of monocytes or histiocytes (for instance, extrafollicular germinal cells of the cortex of the lymph nodes and the Kupffer cells). Therefore, it is evident that there is a similar way of reaction among the precursors of mononuclears.

Finally, it is interesting to consider that these findings, which demonstrate a kind of electivity of antigens for certain undifferentiated or germinal reticuloendothelial cells, seem to accord with the concept that these cells function as producers of antibodies.[2]

2. Bunting, C. H.: Handbook of Hematology, New York, Paul B. Hoeber, Inc., 1938, vol. 1, p. 439. Epstein, E.: Virchows Arch. f. path. Anat. **273**:89, 1929. Bueno.[1]

NUCLEOLAR SUBSTANCE IN THE ANTERIOR LOBE OF THE HUMAN PITUITARY GLAND

CESARE CAVALLERO, M.D.

MILAN, ITALY

A NUMBER of investigators [1] have suggested that both in man and in a number of animals the nuclei of the glandular epithelium of the pituitary gland take part in the process of cellular secretion. Erdheim and Stumme [2] called attention to the presence of huge vesicles of nucleolar origin in the pituitary gland of the pregnant woman. Gellerstedt and Lundquist [3] and shortly afterward Mellgren [4] pointed out peculiar cells in the pituitary body in cases of basophilism and of adrenogenital syndromes. These cells were characterized by an intranuclear acidophilic vesicular formation which at times reached such a size as to almost completely cover the nuclear area. Cells corresponding to the descriptions of these investigators were described by Romeis [1f] as observed in human pituitary glands otherwise considered to be normal.

The results of a further investigation of the intimate cytologic structure of these intranuclear formations, their frequency, their relation to age and to various pathologic conditions and significance are here briefly presented.

MATERIAL AND METHODS

The material submitted to examination consisted of 404 consecutive pituitary glands obtained at postmortem examinations; 200 belonged to males and 204 to females. Persons of all ages were represented in the group, including 10 fetuses from the fourth to the eighth month. Except for 5 normal persons, whose deaths were due to violence, all the persons from whom pituitary glands were taken died of pathologic conditions, some of which involved one or more of the endocrine

1. (a) Pirone, R.: Arch. di fisiol. 2:60, 1905. (b) Guerrini, G.: Sperimentale, Arch. di biol. 58:837, 1904. (c) Trautmann, A.: Frankfurt. Ztschr. f. Path. 18: 173, 1915. (d) Bock, F.: Ztschr. f. Zool. 131:645, 1928. (e) Kirkman, H.: Am. J. Anat. 61: 233, 1937. (f) Romeis, B.: Hypophyse, in von Mollendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1940, vol. 6, pt. 3, p. 597.

2. Erdheim, J., and Stumme, E.: Beitr. z. path. Anat. u. z. allg. Path. 46:1, 1909.

3. Gellerstedt, N., and Lundquist, R.: Upsala läkaref. förh. 45:233, 1939.

4. Mellgren, J.: Beitr. z. path. Anat. u. z. allg. Path. 106:482, 1942.

glands. The material studied is therefore being divided into "endocrine" and "nonendocrine" groups.

The pituitary bodies were removed from five to eighteen hours after death, immediately fixed in 5 per cent formaldehyde solution or in Zenker or Susa solution, embedded in paraffin and cut in sections 5 microns thick, according to a horizontal plane. The sections were stained with hematoxylin-eosin, iron-hematoxylin (Mallory) and cresofuchsin-azocarmine. In a number of cases the study was conducted on serial sections practically including the entire gland. A magnification of 480 to 600 diameters has been found most suitable for the detection of the formations under consideration.

OBSERVATIONS

Cytologic Study.—Vesicular nucleolar-like structures were seen inside the nuclei in 34 per cent of the pituitary glands examined. The cells displaying this peculiar pattern were consistently found in the anterior lobe; they ranged in number from 1 to 5, less frequently from 5 to 10 and in exceptional cases from 50 to 120 per section.

In the sections stained with hematoxylin and eosin these cells (which from now on, for the sake of brevity, will be called X cells) stood out conspicuously because of their notable size and the characteristic globular appearance of the large, intensely acidophilic formation within the nucleus; at other times, however, they could be detected only after careful investigation, as they did not appear larger than the surrounding epithelial cells (figure). In no instance were X cells recognized in the posterior lobe or in the pars intermedia, and most of them appeared to be in the marginal and posterior portions of the anterior lobe, often where this lobe joined the intermediate lobe.

As far as size and shape are concerned, the cells ranged from 10 to 35 microns in diameter and from round to ovoid. The cytoplasm was abundant, spongy in appearance, palely stained and usually amphophilic, with variations from slightly acidophilic to slightly basophilic. In the vacuolated cytoplasm a few thin granules, of rather uniform size, were recognizable. The granules stained pink with eosin, pale yellow with orange G and from gray to violet with the cresofuchsin. The majority of the X cells had characteristics of chromophobe cells; in some cases, however, identical endonuclear inclusions were seen in basophilic degranulated cells and occasionally in typical eosinophilic cells. In pregnancy —also in other conditions showing pregnancy-like changes—endonuclear inclusions were found in pregnancy cells.

The nucleus occupied a good portion of the cell, measuring from 15 to 22 microns in diameter. It varied in shape from round to ovoid, with marked irregularity of the contours. When the nuclear inclusion was small, the chromatin of the nucleus was disposed in a delicate net, thickened at the nodal points and at the inner aspect of the nuclear membrane; however, when the inclusion was so large as to cover most of the nuclear area, the substance proper of the nucleus was reduced

to a narrow rim, and the chromatin appeared in the form of large blocks, well separated one from the other. Cells with two nuclei were encountered occasionally, only one of the two nuclei displaying evidence of nuclear inclusion.

The intranuclear formation itself ranged from 10 to 20 microns in diameter and appeared like a vesicle, clearly outlined, compact and more or less intensely acidophilic, usually homogeneous but sometimes with flakelike or granule-like colorless lacunar spaces. It stained pink with eosin, orange-yellow with orange G and bright red with azocarmine. Thionine and toluidine blue did not stain it metachromatically; it was not stained by mucicarmine; it gave neither the iron
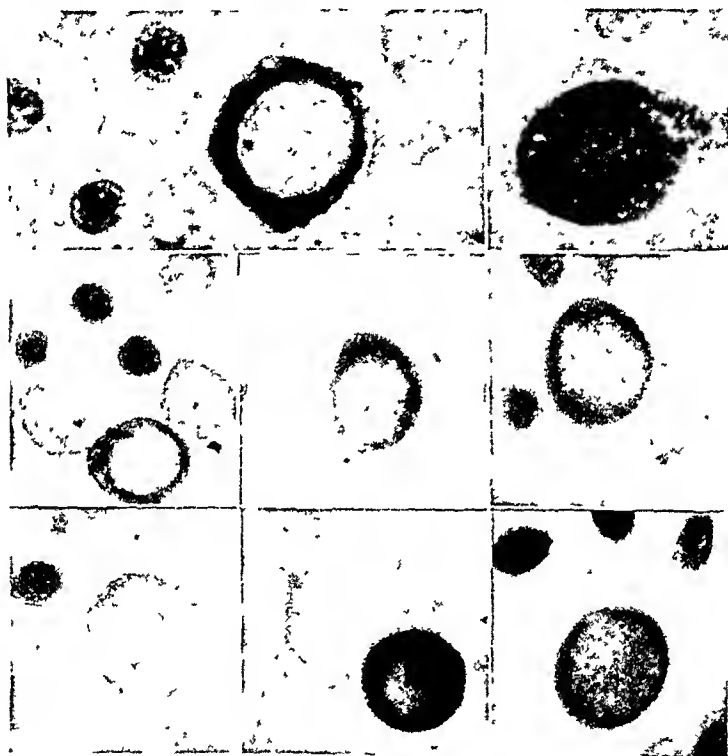
Cells of the anterior lobe of the human pituitary gland with giant nucleoli ("X cells"). Hematoxylin-eosin; × 1,000.

reactions nor those of fats or lipids. Bauer's reaction for the glycoproteins appeared to be only slightly positive.

Relation to Sex and Age.—The X cells are not constantly found in the anterior lobe of the human pituitary body, their appearance showing close relations with age and with certain physiopathologic conditions. The incidence of the finding in the two sexes and in the different age groups is shown in table 1.

In only 34 per cent of the 404 pituitary bodies examined could X cells be detected. No differences were noticed in them between the

two sexes; the slightly higher percentage of females whose pituitary glands contained these cells lacked statistical meaning.

In none of the 51 pituitary bodies of prepubescent persons, including fetal and neonatal specimens, was it possible to detect a single X cell. The highest percentages have been observed at the time of sexual maturity in both sexes. In the climacteric and the postclimacteric period the finding decreased in frequency both in males and in females, to rise again in males beyond the seventh decade of life.

Relation to Physiopathologic Conditions.—The material has been divided into two groups, one including pituitary bodies of persons with endocrine disorders and the other those of persons who did not have endocrine disorders; in the former group those of women who died during pregnancy and puerperium have been included.

TABLE 1.—*Relation of Cells Containing Endonuclear Inclusions to Sex and Age.*

Subjects	Age	Percentage of Males Whose Pituitary Glands Revealed X Cells	Percentage of Females Whose Pituitary Glands Revealed X Cells	Percentage of Total Group Whose Pituitary Glands Revealed X Cells
10...	Under 1 yr.	0	0	0
41...	1-10 yr.	0	0	0
21...	11-20 yr.	35	43	38
25...	21-30 yr.	60	65	64
53...	31-40 yr.	35	56	47
73...	41-50 yr.	37	39	38
80...	51-60 yr.	24	26	25
56...	61-70 yr.	21	22	21
35...	Above 70 yr.	42	4	10
Total 404		33	35	34

A comparison between table 2, in which the "endocrine" group is analyzed, and table 3, in which the "nonendocrine" group is considered, shows a much higher incidence of X cells in the former than in the latter: 65 per cent (68 of 104 studied) in the "endocrine" group, compared with the 23 per cent (40 to 300) in the "nonendocrine" group.

The consistent finding of X cells in pregnancy and puerperium deserves special notice. The material represented primiparas and multiparas and stages from the third month of pregnancy to the fourth day of puerperium; death occurred because of puerperal septicemia, septic abortion, eclampsia, severe hemorrhage occurring after birth, placenta previa or high tracheal stenosis caused by a bulky retrosternal goiter.

The number of X cells seemed to increase with the advance of pregnancy. In the pituitary gland of a woman who died in puerperium four days after childbirth, as many as 100 X cells were found in a single section, and a good number of them appeared to be in the phase of emptying their secretion into the cellular cytoplasm. The intranuclear vesicle seemed to become progressively larger until it came

in direct contact with the nuclear membrane. At a further stage the vesicle was seen to empty its contents into the cytoplasm, this in absence of any evidence of an actual breaking through the nuclear membrane; after excretion of its contents the vesicle assumed a flakelike appearance, losing its individuality.

X cells were also consistently found in the pituitary glands of the 7 females listed as having undergone "female castration." This includes bilateral ovariectomy, total destruction of the ovaries due to bilateral cystic degeneration or to metastatic new growth (Krukenberg's tumor) and severe ovarian hypoplasia. All these patients were in the period of sexual maturity, their ages ranging from 32 to 50 years. X cells

TABLE 2.—*Relation of Cells Containing Endonuclear Inclusions to Endocrine Disorders*

Subjects	Pathologic Condition	Number with X Cells	Percentage with X Cells
19	Pregnancy and puerperium	19	100
7	Female castration	7	100
4	Hypogonadism of male (eunuchoidism)	4	100
1	Gynecomastia	1	100
1	Adrenogenital syndrome	1	100
7	Tuberculosis of adrenal gland with cortical hypofunction (Addison's disease)	7	100
5	Thyrotoxicosis	4	80
4	Hypogonadism of female	3	75
6	Prostatic hypertrophy	4	66
16	Gonadal neoplasms	10	63
8	Diabetes mellitus	2	25
18	Postclimacteric adiposity	5	27
4	Hyperostosis frontalis interna (Morgagni's syndrome)	1	25
4	Myasthenia gravis due to thymic tumor; hypopituitary cachexia (Simmond's disease); diabetes insipidus; pituitary dwarfism	0	0
Total 104		68	65

in great numbers were found in the anterior lobe of the pituitary gland of a 60 year old woman with the adrenogenital syndrome and an ovarian arrhenoblastoma.

The same appeared to be true among the males with gonadal insufficiencies, including eunuchoid adiposity and hypophysial gigantism and, in a single patient, gynecomastia associated with a femininizing neoplasm comparable to arrhenoblastoma. In the last case, that of a 57 year old man, the X cells were scattered in high number throughout the anterior lobe and showed patterns suggesting emptying of secretion.

In Addison's disease X cells were also constantly found but in a limited number; a concomitant numerical decrease and a pronounced degranulation of basophilic cells were noted, and an increase both in size and in number of chromophobe cells.

In 4 of 5 pituitary glands of patients with thyroid conditions X cells were seen; this concerned women between 43 and 68 years of age submitted to hemithyroidectomy or treated with thiouracil. In this group the intranuclear vesicles were so large as to cover almost completely the nuclei of the acidophilic cells.

In the nonendocrine group the highest number of pituitary glands containing X cells came from persons dying of neoplastic disease, especially those with a brain tumor near the sella turcica or in the middle cranial fossa which had caused compression of the pituitary body.

COMMENT

From the foregoing observations it is apparent that the occurrence of the intranuclear formations seen in the glandular epithelium of the pituitary gland is in some way related to age and gonadal function. These formations were not found in the prepubescent period and appeared most numerous, without any significant difference between male and female, in the period of sexual maturity and in a number

TABLE 3.—*Relation of Cells Containing Endonuclear Inclusions to Nonendocrine Conditions*

Subjects	Condition	Number with X Cells	Percentage with X Cells
49	Intracranial tumor	27	55
94	Neoplasms .	30	32
17	Leukemia..	4	24
3	Splenectomy ..	2	66
32	Vascular hypertension	2	6
97	Other diseases	5	5
8	Normal . ..	0	0
Total 300		70	23

of endocrine disorders interfering with gonadal function. They were found at times in chromophilic cells, at other times in chromophobe elements with pregnancy or pregnancy-like characteristics. Gellerstedt and Lundquist[3] and Mellgren,[4] in their descriptions of hypertrophic chromophobe cells with giant nucleoli in cases of Cushing's disease (pituitary basophilism) and adrenogenital syndromes, suggested a possible relation with the "hyaline change" of the basophilic cells. That a connection existed between Crooke's hyalinosis and the X cells of my series is not likely; nevertheless, giant nucleoli often appeared in epithelial elements in the process of progressive degranulation, making identification of the hypophysial cell type extremely difficult and sometimes impossible.

In the light of the observations made by Caspersson and Santesson[5] pointing to heterochromatin (the substance entering into the constitu-

5. Caspersson, T , and Santesson, L.· Acta radiol , 1942, supp. 46.

tion of nucleoli) as the most important center of the protein syntheses and protein exchanges between nucleus and cytoplasm, the unusual richness in heterochromatin of the X cell might be considered as evidence of cellular hyperfunction.

A finding similar to that noticed in the X cells of the pituitary gland has been demonstrated in the hepatic cells of man and animals by Berg,[6] in the pineal body by Krabbe,[7] Volkmann[8] and Meyer,[9] in the cells of the pars intermedia of the pituitary gland of the mouse by Urasov[10] and Romeis,[1f] in the cells of the cortex of the adrenal gland by Schiller[11] and in the pituicytes of the human neurohypophysis by Bargmann.[12] The nucleolar patterns observed in these different structures have been generally explained on the basis of nuclear secretory activity. For the finding, closely resembling these, in the human pituitary gland, I incline toward a similar interpretation. This interpretation is further strengthened by patterns indicating a periodical emptying of the nuclear inclusion into the cytoplasmic substance. In conclusion, it is suggested that these intranuclear formations are of nucleolar origin and that they represent morphologic evidence of nuclear secretory activity.

SUMMARY

Intranuclear formations observed in the glandular cells of the human pituitary gland are described and analyzed with respect to their morphologic aspects, frequency, relation to age and disease processes, and significance.

Four hundred and four pituitary glands were examined, and evidence of such formations was found in 34 per cent. As far as sex is concerned, no differences were seen in these structures between males and females; definite relations were noticed instead with respect to age and gonadal function. The intranuclear bodies were found in both the chromophilic and the chromophobe cells but most frequently in hypertrophic chromophobe elements with pregnancy or pregnancy-like characteristics. It is suggested that these formations are of nucleolar origin and that they are the product of nuclear secretion.

6. Berg. W.: Ztschr. f. mikr.-anat. Forsch. **35**:146, 1934.

7. Krabbe, H.: Anat. Hefte, 1916, no. 54, p. 190.

8. Volkmann, R.: Ztschr. f. Neurol. **84**:593, 1923.

9. Meyer, R.: Ztschr. f. Zellforsch. u. mikr. Anat. **25**:614, 1937.

10. Urasov, I.: Russk. Arch. Anat. **6**:149, 1927.

11. Schiller, E.: Ztschr. f. mikr.-anat. Forsch. **54**:598, 1944.

12. Bargmann, W.: Ztschr. f. Zellforsch. u. mikr. Anat. **32**:394, 1942.

Laboratory Methods and Technical Notes

IMPROVED METHOD FOR HANDLING PHOTOLABILE LIQUIDS

JOHN NICHOLS

CHAPEL HILL, N. C.

ALL USERS of photolabile liquids such as silver nitrate and osmic acid recognize the problem of preventing the deterioration due to light. Most laboratory texts say that these liquids should be kept in amber or dark-colored bottles and stored away from the light. However, if these directions are followed, there is still no certainty as to the extent of deterioration of the liquid. Important experiments may be
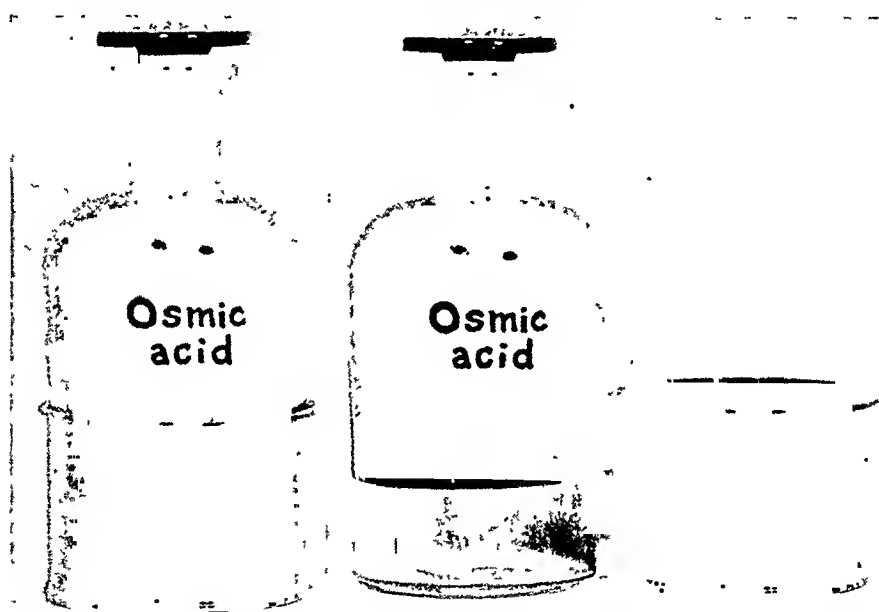
Painted bottle and cup for preventing deterioration of a stored photolabile liquid.

ruined as a result. The only alternative is to store the liquid in a clear glass bottle and keep it in a dark cabinet. This has many obvious disadvantages, although the condition of the liquid can be determined at a glance.

A device has been tried which seems to surmount most of the difficulties. A brief description follows.

A convenient-sized bottle with a mushroom stopper is painted jet black with a paint such as Fisher's "plicote." Care is taken not to paint any of the surface at the mouth of the bottle to avoid contaminating the liquid. A strip of adhesive tape is applied to a height of about 1 inch (2.5 cm.) from the base of the bottle

---

From the Department of Anatomy, University of North Carolina.

to avoid painting this area and to make a sharp border. On removal this leaves a portion of clear glass through which the liquid can be viewed. A small metal can similar to a drinking cup, which may be obtained from a 10 cent store, is also painted jet black inside and out. When the bottle is placed in the cup, after drying, there should be at least an inch of overlapping painted surfaces (figure).

The advantages offered are: (1) No light can penetrate the liquid, since the cup need not be detached from the bottle during pouring, and (2) the contents can be viewed at will by merely lifting the bottle from the cup.

General Reviews

MECHANISMS OF ABNORMAL DEVELOPMENT
III. Postnatal Developmental Abnormalities

PETER GRUENWALD, M.D.
NEW YORK

(Concluded from Page 559)

IN the introduction to this review the fact was emphasized that no strict separation exists between developmental abnormalities of early life, on the one hand, and many of the pathologic conditions of the mature organism, on the other. One might contend that some measure of development is involved in all pathologic changes of the structure of the body, and thus bring morphologic pathology almost entirely into the scope of developmental pathology. However, the present part of this review will be confined to some of the more obvious deviations of postnatal development. It will not include those subjects which are customarily treated in a systematic manner in textbooks—for example, the structural changes resulting from inflammation or from the action of hormones. An attempt will be made to cite examples which are comparable to embryonic abnormalities mentioned in the preceding parts, in order to establish a link between the developmental pathology of the embryo and that of the adult, which is much better known. Usually they are considered from different points of view.

All those causes of structural changes which were discussed in part I with particular reference to the embryo are also effective during postnatal life. However, their relative importance is different in the two periods of life. After birth the action of intrinsic (genetic) causes, while still demonstrable in many instances, is overshadowed by that of extrinsic agents, such as mechanical forces, chemical substances, actinic rays or infectious organisms. Similarly, the mechanisms of correlation by which multiple effects are produced after one initial lesion of the

embryo (see part II) are all still in existence after birth, though not with the same relative significance.

During normal development there is a gradual shift in the extent to which various correlations are active; this is reflected in a similar shift of the mechanisms of abnormal development. After birth, the basic developmental pattern of the organism is no longer subject to changes, and developmental correlations—for example, induction—are active only in the limited spheres of tissue differentiations. Genetic control of structure exists at any time, but it is not so obvious after birth. On the other hand, correlations based on organic functions, particularly those of the nervous systems and the endocrine glands, gain greatly in significance. The space allotted to the effects of various agents and correlations in the following pages will not reflect their relative significance, as those changes will be discussed in detail which are less known and which resemble the ones that are more prominent in the embryo.

HEREDITARY ABNORMALITIES

Many of the abnormal hereditary traits appear after birth, and with all methods at one's command it is impossible to detect in the newborn an abnormality which might have developed before birth. Examples to illustrate this will be cited here with the reservation that there may have been failures to discover prenatal changes. This consideration and the fact that the abnormal genotype is present in the organism at all times, no matter when it manifests itself structurally, show that there is no essential difference between hereditary changes appearing during various periods of life.

The purely descriptive literature on many of the hereditary conditions in man will not be reviewed. It may be found through references in texts of human genetics.[45]

Reference has been made previously in this review to the fact that even in the well protected embryo the manifestation of hereditary traits may be influenced by the environment. This holds, of course, to a much greater extent for postnatal life. The modifying effects of various extrinsic factors may be so great that the hereditary background is considered as merely a "disposition" toward a certain change—for example, diabetes mellitus.

Many hereditary abnormalities manifest themselves by degenerative changes which appear at a characteristic time, sometimes late in life. These have been termed heredodegenerative diseases. They are closely related to obvious malformations. It is known that such congenital defects as taillessness in mice develop in the early phase of the embryo by a very similar process of degeneration of tissues which were previously normal in appearance.

Skin.—The morphologic expression and the morphogenesis of hereditary hairlessness of man and other mammals have been examined and the literature reviewed by David.[282] The condition may appear before or after birth, depending on the genotype. Among mice with one form of dominant hairlessness, homozygous animals can be recognized at birth, whereas heterozygous animals do not appear abnormal at birth, their abnormality developing later on. According to the histologic changes, David distinguishes three forms of hypotrichosis, namely, *hypokeratotica, cystica* and *hypoplastica follicularis.* For details the original article should be consulted. Whether human baldness is comparable with these findings in animals is questionable. There seems to be a hereditary disposition, and, in addition, an influence of androgenic hormone.[498] Among the hereditary postnatal cutaneous diseases which show more or less clearly the character of developmental abnormalities are xeroderma pigmentosum, neurofibromatosis and psoriasis.

Nervous System and Sense Organs.—In the case of some abnormalities of the brain it is particularly difficult to determine the onset of abnormal changes in relation to birth, because many of the functions of that organ cannot be tested in the newborn.

Retardation or arrest of development occurs after birth in mongolism.[499] However, other manifestations of this condition date back to prenatal life.

Qualitatively abnormal development, producing a tissue structure different from any normal stage, occurs in tuberous sclerosis,[500] amaurotic familial idiocy [501] and other diseases. The exact time of onset is not known.

Numerous examples of heredodegenerative conditions of the nervous system are on record. Among these are paralysis agitans,[502] hereditary sclerosis, spinal form (hereditary ataxia, Friedreich's ataxia), hereditary chronic progressive chorea (Huntington's chorea), hepatolenticular degeneration (Wilson's disease),[45] loss of coordination in rabbits [503] and many others which may be clinically more important but which have not been thoroughly studied from the pathologic and the genetic point of view.

498. Hamilton, J. B.: Am. J. Anat. **71**:451, 1942.

499. Benda, C. E.: Am. J. Ment. Deficiency **45**:42, 1940.

500. Globus, J. H., in Penfield, W.: Cytology and Cellular Pathology of the Nervous System, New York, Paul B. Hoeber, Inc., 1932. Yakovlev, P. I., in Blumer, G.: The Practitioners Library of Medicine and Surgery, New York, D. Appleton-Century Company, Inc., 1936, vol. 9, p. 745.

501. Globus, J. H.: J. Mt. Sinai Hosp. **9**:451, 1942.

502. Allan, W.: Arch. Int. Med. **60**:424, 1937.

503. Anders, M. V.: Am. J. Anat. **76**:183, 1945.

Several forms of hereditary metabolic deficiency affect the nervous system—for example, amaurotic familial idiocy and phenylketonuria. In the former the nerve cells accumulate the abnormal metabolic product; in the latter the mechanism by which the brain is affected is not understood.

The sense organs present excellent examples of hereditary postnatal maldevelopment. In the eyes of mice examined by Keeler [341] the differentiation of the retina was arrested shortly after birth, and this led to complete or partial absence of rod cells. A somewhat similar end result has been found in a strain of rats after degeneration of the previously well differentiated retina.[504] The latter strain, as well as a mutant of mice described by Grüneberg,[1r] shows a high incidence of cataract developing after birth.

In man, retinitis pigmentosa develops as a degeneration of the retina [505] at a varying age, depending on the mode of inheritance in the particular family.[506] Certain forms of atrophy of the optic nerves, cataract and glaucoma are also hereditary.[45]

Postnatal degenerative changes of the labyrinth account for deafness and other abnormalities of labyrinthine function in certain strains of mice. In shaker-1 mice the stria vascularis is abnormal, and as soon as the vas spirale atrophies in the usual manner, Corti's organ degenerates because it is not adequately supplied with endolymph from the stria vascularis.[507] In another mutant, the "waltzing" mouse, the inner ear is normal at birth. There is disagreement as to whether in this form there is also primary degeneration of the stria vascularis with consecutive damage of the labyrinth or primary degeneration of the ganglion spirale cochleae or of the acoustic tracts of the brain.[508]

In man a hereditary change which involves abnormal development is otosclerosis. The morphogenesis and the genetics of this condition have been reviewed in detail by Bauer and Stein.[509] Abnormal bone is formed and replaces normal bone; it forms in excessive amounts and causes fixation of the stapes. In addition there are degenerative changes in the labyrinth and its nerves. The relation of these changes to the abnormal growth of bone is controversial. Bauer and Stein conclude from investigations of the familial occurrence of diseases of the inner ear that there is a hereditary inferiority of the organ (*Organminderwer-*

504. Bourne, M. C., and Grüneberg, H.: J. Hered. 30:130, 1939.

505. Friedenwald, J. S., and Chan, E.: Arch. Ophth. 8:172, 1937.

506. Allan, W.: Arch. Ophth. 18:938, 1937.

507. Grüneberg, H.; Hallpike, C. S., and Ledoux, A.: Proc. Roy. Soc., London, s.B 129:154, 1940.

508. Grüneberg,[1r] p. 130.

509. Bauer, J., and Stein, C.: Konstitutionspathologie in den medizinischen Spezialwissenschaften, Berlin, Julius Springer, 1926, no. 2.

tigkeit), transmitted by a combination of two recessive genes. This inferiority may manifest itself either as otosclerosis or in the form of purely degenerative diseases. The histologic changes of otosclerosis have been found in cretins and deaf-mutes at a very early age.

Teeth.—The teeth present an excellent opportunity to study postnatal developmental disturbances because much of their complex development takes place after birth. Identical changes may originate before and after birth, and for this reason all malformations of teeth' will be discussed jointly in various sections of this part of the review.

Weinmann [510] has classified hereditary abnormalities of enamel formation according to the developmental process involved. If the deposition of enamel matrix is inhibited, hypoplasia of enamel results; if the maturation is affected, hypocalcification is seen. Both abnormalities may also result from other than hereditary causes. Weinmann points out that the hereditary forms affect all teeth uniformly and regardless of the time of the formation of these, whereas systemic forms, caused by metabolic or endocrine disturbances, as well as those due to localized trauma, are limited to the portions of the enamel which develop at the time and the place affected.

In the mutation gray-lethal of mice, with skeletal changes which have been referred to in part II, severe abnormalities of tooth development and eruption are apparently due to a lack of bone resorption in the jaws which would normally provide the necessary space.[511] A somewhat similar hereditary malformation occurs in rats.[512] At birth the animals appear normal. Shortly after birth spicules of bone begin to encroach on the apical portions of the incisor and first molar teeth and cause severe deformity and ankylosis of the tooth germs. The incisor teeth develop into large, unerupted, tumor-like masses of dental tissues. The first molar teeth show a lesser degree of abnormality, and the second and third molar teeth, which develop later, are more nearly normal. This points to a selective action of the abnormal trait beginning shortly after birth. Only preliminary studies have been published as yet.

Skeleton, Muscles and Tendons.—Various skeletal malformations which begin to manifest themselves in the embryo (see part II) not only show after birth the effect of the prenatal abnormality but continue to follow a devious course of development. These include, among others, various forms of chondrodystrophy and the gray-lethal malformation of mice.

510. Weinmann, J. P.: Bur **43**:20, 1943.

511. Grüneberg, H.: J. Anat. **71**:236, 1937.

512. Schour, I.; Massler, M., and Greep, R. O.:' J. Dent. Research **23**:194, 1944.

A generalized malformation of cartilage which appears after birth is hereditary in the rat.[513] There is excessive growth of cartilage, and the resulting changes in the trachea and the ribs lead to emphysema and fatal pulmonary complications. Experimental transplantations have shown that the property is inherent in the tissue: grafts of cartilage from abnormal rats develop abnormally in normal hosts, and vice versa.

Postnatal hereditary abnormalities of the human skeleton have been reviewed by Aschner and Engelmann.[372a] Examples are: Legg-Calvé-Perthes disease (osteochondrosis of the head of the femur) and osteopsathyrosis (fragilitas ossium). In the muscle system there are such heredodegenerative changes as progressive muscular atrophy and peroneal atrophy (progressive neuropathic muscular atrophy). Concerning tendons, Dupuytren's contracture of the palmar fascia might be mentioned.

Other Organs.—Imperforate vagina occurs as a hereditary trait in mice.[1r] The abnormality manifests itself at puberty, when the normal vagina acquires a lumen. This process fails in the abnormal mice, and it cannot be initiated by estrogenic treatment. If the vagina is opened surgically, the female is fertile. However, the vagina retains a tendency to close, in contrast to the normal vagina, which tends to open again if it is closed surgically.

Another strain of mice shows, beginning at the age of 3 to 4 months, severe adenomatous thickening of the mucosa of the pyloric portion of the stomach.[514] This observation leads into a field which should be of considerable medical importance, namely, hereditary differences in the old age changes of various organs. The following examples are discussed more in detail in Grüneberg's book on genetics of the mouse.[1r] Gorer [515] found in each of three strains of mice which he examined a specific change in the kidneys in advanced age. One strain showed metaplasia of the parietal layer of Bowman's capsule more frequently than did other mice. In another strain necrotic lesions of the papillae appeared, with consecutive dilatation of tubules. The third strain showed hyaline degeneration of the connective tissue framework. It is highly significant that these changes were not the result of diseases caused by extrinsic agents but were rather the expression of the genetic constitution, either alone or, as a predisposing factor, in combination with extrinsic influences which would otherwise not have this effect. The changes just mentioned are obviously related to the so-called heredodegenerative diseases which have just been referred to and to prenatal malformations which develop by degeneration of previously normal-appearing parts.

513. Grüneberg, H.: Proc. Roy. Soc., London, s.B **125**:123, 1938. Engel, S., and Grüneberg, H.: J. Genet. **39**:343, 1940.

514. Andervont, H. B., and Stewart, H. L.: Science **86**:566, 1937. Stewart, H. L.: J. Nat. Cancer Inst. **1**:489, 1941.

515. Gorer, P. A.: J. Path. & Bact. **50**:25, 1940.

There are interstrain differences in the occurrence of the so-called brown degeneration of the adrenal glands and in the structure of the follicles of the thyroid gland and the incidence of goiter. Also in mice there are genetically determined differences in the time of appearance of old age changes of bones and joints.[516]

Progressive facial hemiatrophy of man has been considered by Wartenberg [65d] as a heredodegeneration, possibly mediated by unilateral changes in the nervous system.

MECHANICAL AGENTS

Mechanical injury in the widest sense accounts for many structural changes. In most cases the abnormality is just the result of an elimination or a disfiguration of parts plus the usual processes of wound healing; these cases offer nothing to be discussed here. There are, however, instances in which more complex developmental processes are modified by mechanical injury. Among these is the growth of tissue displaced by trauma.

Few noncancerous tissues of the mature organism grow for a long period when transplanted to abnormal locations, unless special precautions are taken as in surgical grafting. Several cases are on record in which multiple nodules of splenic tissue grew on the peritoneum after the spleen had suffered traumatic rupture.[517] Another example, namely, endometriosis, is controversial. Some authors assume that viable endometrial tissue is displaced either by being regurgitated through the tubal ostiums or in the course of surgical procedures [518] as suggested by the appearance of endometriosis in surgical scars. On the other hand, there are sites of endometriosis which are not explained by either of these theories. It has been demonstrated that all known locations of endometriosis can be accounted for embryologically without recourse to mechanical displacement (see part II). This, of course, does not disprove the claim that mechanical transportation of tissue has occurred in some cases.

It may appear strange at the first glance that bone, one of the tissues of the body which mechanically are most rigid, should react most actively to mechanical influences by developmental changes. Yet it is this very rigidity which makes developmental changes of the structure necessary in cases in which other tissues, such as muscles, tendons or ligaments, could adapt themselves by means of their flexibility. The arrangement of the trabeculae and the haversian systems of bone is adapted to mechanical

---

516. Silberberg, M., and Silberberg, R.: Am. J. Anat. **68**:69, 1941.

517. Buchbinder, J. H., and Lipkoff, C. J.: Surgery **6**:927, 1939. Jarcho, S., and Anderson, D. H.: Am. J. Path. **15**:527, 1939.

518. Sampson, J. A.: Arch. Surg. **3**:245, 1921. Wespi, H. H., and Kletzhändler, M.: Monatschr. f. Geburtsh. u. Gynäk. **111**:169, 1940.

strain, and any change occurring in the mechanical conditions of the environment is immediately followed by resorption and rebuilding of parts of the bone. This is well demonstrated by the minute structure of bones with abnormal curvatures.[519] Pressure causes resorption of bone not by crushing or destroying the tissue but by the action of osteoclasts. This explains, for example, why the bodies of vertebrae are less resistant to the pressure exerted by an aortic aneurysm than are the intervertebral disks. Mechanical pull, on the other hand, causes overgrowth of bone, as seen at the points of insertion of tendons.

Müller [520] has presented in book form the developmental physiology and pathology of bone, with particular reference to the differentiation of inherent and extrinsic factors of development. Iselin [521] has pointed out how the principles of developmental mechanics should be used as a basis for rational therapy in orthopedics.

Displaced tissues of the mature organism may in a few instances act on their new surroundings as inductors. The best studied example is that of bone formation inducted by transplants of mucosa of urinary passages.[454] It has been found that the connective tissue reacts by formation of bone only in certain locations (e. g., the abdominal wall). In other locations (e.g., the liver, the spleen, or the wall of the stomach) a connective tissue capsule develops around transplants of bladder mucosa, but no bone. This has been compared with similar observations in embryologic experiments demonstrating that only part of a morphologically uniform tissue is able to react to certain inductions.[522] The fact that alkaline phosphatase is present in the transplanted epithelium has led to the suggestion that phosphatase diffusing into the connective tissue might be the cause of ossification. This has been ruled out not only by the negative findings in certain areas, even though connective tissue was present and the epithelium contained phosphatase, but also by the negative results observed in all locations of other transplanted phosphatase-containing epitheliums. The phosphatase which is present in areas of ossification induced by bladder mucosa is produced by fibroblasts as the first known response to the induction, before routine staining methods show any change in these cells.[522]

THE INFLUENCE OF CHEMICAL AGENTS

Certain substances which may or may not be essential for normal development and function in certain amounts are poisonous when present

519. Landauer, W.: Arch. f. Entwcklngsmechn. d. Organ. **115**:911, 1929. Sternberg, H.: Ztschr. f. orthop. Chir. **63**:387, 1935. Murray, P. D. F.: Bones, London, Cambridge University Press, 1936.

520. Müller, W.: Die normale und pathologische Physiologie des Knochens, Leipzig, Johann Ambrosius Barth, 1924.

521. Iselin, H.: Schweiz. med. Wchnschr. **14**:465, 497 and 536, 1933.

522. Gomori, G.: Am. J. Path. **19**:197, 1943.

in excessive amounts. In many instances their action, though morphologically specific to some degree, is of little interest from the standpoint of this review. Among those substances which have a definite influence on development are carcinogens, which will be mentioned in a later section dealing with cancerous growth, as well as others which affect normal developmental processes. In addition, deficiencies of some inorganic compounds may disturb development.

Other groups of substances are specifically related to developmental processes. Vitamins are essential for normal development; their lack or, in rare cases, their excess causes disturbances. Hormones are elaborated in the body, and one of their principal functions is the control of certain phases of morphogenesis (see also parts I and II). It is obvious that excess or lack of these "messengers" will profoundly influence development and maintenance of structure. Most of the well studied morphologic sequelae of abnormal levels of hormones and vitamins have been treated systematically in many monographs and textbooks and will therefore not be dealt with here. Only a few examples will be given.

The continuously growing teeth of rodents are a good object for the study of the effect of all of the aforementioned types of chemically caused disturbances. The formation or the maturation of the enamel, the formation of dentin, the eruption of the tooth and the structure of the socket may be affected singly or in various combinations.

Deficiency of magnesium in the diet of rats reduces the rate of eruption. Dentin formation is also reduced, and ceases completely in focal areas.[523] An excess of fluorine compounds reduces the rate of eruption and causes defective calcification of dentin and enamel. If large doses are given, enamel formation suffers by a shortening of the appositional life span of the ameloblasts. Changes observed in human fluorosis are in some respects similar to these experimental results.[524] Strontium and manganese compounds cause hypoplasia of enamel but in somewhat different manners, as has been shown by Wessinger and Weinmann[525] in histologic studies and illustrative diagrams.

The effects of vitamin deficiencies on postnatal developmental processes in man and in experimental animals has been summarized by Wolbach and Bessey[526] in a review in which much information and a comprehensive list of references may be found. A smaller volume of data on hypervitaminoses is also contained in that review. At an earlier date, Wolbach[527] pointed out that studies of vitamin deficiencies "may be

523. Gagnon, J.; Schour, I., and Patras, M. C.: Proc. Soc. Exper. Biol. & Med. **49**:662, 1942.

524. Schour, I., and Smith, M. C.: Publication 19, American Association for the Advancement of Science, 1942, p. 32.

525. Wessinger, G. D., and Weinmann, J. P.: Am. J. Physiol. **139**:233, 1943.

526. Wolbach, S. B., and Bessey, O. A.: Physiol. Rev. **22**:233, 1942.

527. Wolbach, S. B.: Science **86**:569, 1937.

of value as premises in problems, hitherto approached only by the methods of experimental embryology . . . ". Wolbach and Bessey classify the morphologic manifestations of vitamin deficiencies as follows: (1) diffuse consequences expressive of inanition; (2) effects common to several deficiencies, especially degenerations of the nervous system and, with qualifications regarding fine details, lesions of the skin; (3) degenerative changes characteristic in kind and distribution, best illustrated by the cerebral lesions of thiamine deficiency and the degeneration of skeletal muscles and embryonal tissues observed in vitamin E deficiencies; (4) initial specific effects exhibited by striking changes of structural patterns, outstanding in relation to vitamins A, C (ascorbic acid) and D. As was found earlier in the present review, postnatal maldevelopment is particularly obvious in the skeleton and the teeth, and much of present information on vitamin deficiencies concerns these structures.

One of the most important effects of vitamin A deficiency is seen in the keratinizing stratified epithelium formed in many mucous membranes which normally have a different lining. In the continuously growing teeth of rodents there are atrophy and keratinizing metaplasia of the enamel epithelium with consecutive irregularity, and finally cessation of dentin formation. If the vitamin deficiency is not complete, irregular remnants form tumor-like aggregates of dental tissues.[528]

According to Johnson,[529] vitamin A deficiency produces in the eyes of rats degeneration of the retina and rosette formation. This is of particular interest because, as has been reviewed in part II, rosettes develop in the embryo as abnormalities during the differentiation of the retina. They can apparently also arise secondarily after normal differentiation has been completed. The mechanism is probably one of rearrangement of the remaining cells after extensive degeneration.

Skeletal growth and development are inhibited in avitaminotic young animals, and the ensuing limitation of space in the cranial cavity and the spinal canal causes herniations and other disturbances of the nervous system. An excess of vitamin A produces osteoporosis and decalcification of bone, leading to multiple fractures. Osteoporosis is most marked in areas in which bone is normally being remodeled at the time of the disease.[526]

Deficiencies of vitamins of the B complex are not followed by such characteristic and generalized changes as is exemplified by the epithelial metaplasia of vitamin A deficiency. Most of the pathologic aspects are those of degeneration, with which one is not concerned here. Examples of abnormal development that probably is a consequence of such degen-

---

528. Burn, C. G.; Orton, A. U., and Smith, A. H.: Yale J. Biol. & Med. **13**:817, 1941.

529. Johnson, M. L.: J. Exper. Zool. **81**:67, 1939.

erative changes are imperfect growth of hair, the hyalinization and vascularization of the tunica propria of the cornea seen in riboflavin deficiency and the cirrhosis following fatty changes of the liver in choline deficiency.[526]

Vitamin C produces a characteristic generalized change in the supporting tissues by a "failure of formation and maintenance of intercellular materials".[526] All the changes found in human scurvy and in deficiency experimentally produced in the guinea pig are explained on this basis. The viability of the cells of the affected tissues is not diminished. The formation of bone and dentin is abnormal and finally ceases. Osteoblasts and odontoblasts become indistinguishable from fibroblasts. Wound healing is poor. Wassermann [530] shows that in the teeth of guinea pigs enamel formation ceases in areas exactly corresponding to those of absence of dentin, and he explains this by stating that the ameloblasts depend on the presence of dentin for their normal function.

According to Wolbach and Bessey, vitamin D deficiency acts not directly on the bony structures which show the striking effects but on the absorption of calcium and phosphate, which becomes inadequate for the proper calcification of tissues. This affects the cartilage of the epiphyses of growing bones and thus prevents the normal changes preceding the destruction and bony replacement of that tissue. Consequently the cartilage accumulates in the epiphyses. Another feature of rickets is the deposition of much uncalcified osteoid tissue instead of bone and the resorption of some of the preexisting bone. Osteoid tissue is highly resistant to osteoclastic resorption. In older individuals, in whom epiphysial growth no longer occurs, bone tissue is gradually replaced by solid masses of soft osteoid tissue (osteomalacia). The changes· in teeth have repeatedly been examined. Weinmann and Schour [531] report on rachitic changes of the continuously growing teeth and the alveolar bone of rats and on their modification by various agents. They found, in contrast to some earlier investigators, that the formation and the maturation of enamel were unaffected. The formation and the calcification of dentin were retarded. In addition there were qualitative abnormalities in the dentin. The alveolar bone showed the well known lack of calcification of newly formed bone tissue and failure of this osteoid tissue to be resorbed. This produced a distorted pattern of growth.· The increased resistance of osteoid tissue to resorption remained in evidence after treatment with parathyroid extract. As in other instances, the rachitic changes were reversed by starvation or by the administration of sodium phosphate solution or· viosterol.

---

530. Wassermann, F.: J. Dent. Research **23**:463, 1944.

531. Weinmann, J. P., and Schour, I.: Am. J. Path. **21**:821, 833, 857, 1047 and 1057, 1945.

One of the principal fields of hormone action is the regulation of morphogenesis and structure. The amount of work done in this field is so large, and it has so often been reviewed, that it will not be treated here. Texts of physiology or pathology, as well as specialized accounts of endocrinology, are available for reference. Endocrine influences on tooth development which are not described in detail in many of these works have been reviewed by Schour and Massler [532]; references to original reports may be found there.

A field in which development under hormonal control is particularly active after birth is that of the sex organs and sexually differentiated traits. This is also of great interest, because it demonstrates the interaction of genetic and hormonal controls of development. Certain abnormalities, such as those induced by hormones released by adrenal cortex tumors, are essentially similar before and after birth. Some fundamental aspects of these problems have been discussed in parts I and II.

Inductors are substances of eminent importance in structural development. They differ from hormones in the manner in which they reach their destination in the body: They are directly transmitted from one tissue to an adjacent one and are not distributed to the entire body by way of the blood stream. Abnormal induction will therefore occur when tissues are in contact with one another in a manner in which they would not be in the normal body. This may be brought about by mechanical dislocation of the inductor or of its substrate, as has been illustrated in a previous section with reference to bone induced by transplants of bladder mucosa.

THE EFFECTS OF INFECTION

I shall not discuss here the effects of protracted infectious diseases on general growth and development. The impairment of these processes is not so much a specific effect of the infectious agent as the result of nonspecific unfavorable circumstances, such as malnutrition or fever.

If infection provokes the growth of a tissue which would not usually be present, this is usually in the form of a granulation tissue. A discussion of inflammation and the formation of granulation tissue is not within the scope of this review, even though certain developmental mechanisms are involved in the differentiation of cells in this process and in the determination of the pattern of the lesion. However, brief mention should be made of what has sometimes been called specific granulation tissue. Granulomas of a definite and complex structure are in many cases so characteristic of a causative agent that their presence is considered sufficient evidence for diagnosis in routine practice

---

532. Schour, I., and Massler, M.: J. Am. Dent. A. **30**:595, 763 and 943, 1943.

of pathology, without the need for demonstrating the causative agent itself. The question thus arises whether these granulomas should be considered as specific developmental responses to the presence of certain micro-organisms. If one analyzes these granulomas, one finds that they are composed of a few elements of nonspecific reaction—for example, to foreign bodies. Various combinations of these elements and the peculiar distributions and biologic properties of the micro-organisms involved account for the characteristic appearances which granulomas may have. This has been analyzed in great detail in the case of tuberculosis, where various fractions of the substance of the bacilli have been isolated and the reactions of mammalian tissues to their presence observed. This work, as well as a large volume of information concerning tissue reactions *to various injurious agents*, has been reviewed by Forbus.[533]

In a few instances infection provokes structural alterations which do not fall into the group of inflammatory reactions. One example is the contagious pulmonary adenomatosis of sheep (jagziekte); the exact nature of the infectious agent has not been determined.[534] The lungs show an adenomatous nodular growth of columnar epithelium lining the spaces.

In some persons the teeth show evidence of aplasia of the enamel during a limited period of development, and it has been suggested many times that various diseases, among them infections, may be the cause. Sarnat and Schour [535] found no definite evidence of infectious diseases as the cause of chronologic aplasia of enamel in a series of 60 cases, but they admit that this relationship may occasionally exist. The effect on the teeth is a nonspecific one and does not indicate that the dental tissues were actually infected. The same probably holds for the observations of Kreshover,[536] who reported that in experimental tuberculosis of laboratory animals changes occur similar to the aforementioned chronologic aplasia in man.

DEVELOPMENTAL ASPECTS OF CANCER

The most striking peculiarity of cancer is a developmental abnormality concerning its structural differentiation as well as its rate of growth and its relation to adjacent structures. Many data relating to these and other aspects of the problem of cancer have recently been reviewed by Furth,[66b] and many original reports, as well as previous and more specialized reviews, are listed there.

533. Forbus, W. D.: Reaction to Injury: Pathology for Students of Disease Based on the Functional and Morphological Responses of Tissues to Injurious Agents. Baltimore, Williams & Wilkins Company, 1943.

534. Dungal, N.: Am. J. Path. **22**:737, 1946.

535. Sarnat, B. G., and Schour, I.: J. Am. Dent. A. **28**:1989, 1941; **29**:67, 1942.

536. Kreshover, S. H.: J. Dent. Research **21**:27, 1942; **23**:231, 1944.

Investigators know that all types of causes of abnormal development which have been discussed with regard to embryonic malformations in part I of this review may also produce cancer under proper conditions. They include hereditary factors and influences of the environment of a mechanical, chemical, radiant, thermic or infectious nature. As is often the case in embryonic maldevelopment, several of these factors (e.g., hereditary and environmental ones) may combine their effects also in carcinogenesis.

Hereditary factors may appear in two ways. In one of these, cancer develops in individuals carrying a certain gene or combination of genes either by virtue of these genes alone or when an environmental agent is added. In the latter event the genetic constitution determines what is often called the susceptibility to the environmental agent. The second way in which genes are concerned with cancer relates more directly to the cancerous tissue itself; this is somatic mutation. The fact that the essential abnormality is apparently passed on from one cancer cell to its descendants without the necessity of the continued presence of a carcinogenic agent has led many workers [537] to the conclusion that cancer cells are genetically different from the cells of their host, which constitutes a somatic mutation. In support of this hypothesis it has been pointed out that agents which are known to increase the mutation rate (e.g., roentgen rays) are also carcinogenic. Strong [68c] reports that one of the most commonly used chemical carcinogens, 20-methylcholanthrene, also increases the rate of germinal mutations.

Among environmental agents, virus infection has been held by Oberling [538] to be responsible for all cancers. Actually, it has been found in regard to certain cancers of the mammary glands of mice, which were previously believed to be purely hereditary, that one of the causative agents is a substance transmitted to the young by the mother's milk.[539] The properties of this substance resemble those of a virus as far as they are known. However, Oberling's theory has not been accepted to its full extent.

The nature of the basic abnormality of cancerous growth has been defined in various manners, each depending largely on the line of approach of the investigator. Pathologists, biochemists, immunologists, geneticists and others have found differences between cancerous and noncancerous tissue. Within the scope of the present review are only

---

537. (*a*) Bayne-Jones, S., and others: Pub. Health Rep. **53**:2121, 1937. (*b*) Berrill, N. J.: Physiol. Rev. **23**:101, 1943. Furth.[68b]

538. Oberling, C.: The Riddle of Cancer, New Haven, Yale University Press, 1944.

539. Bittner, J. J., in Research Conference on Cancer, American Association for the Advancement of Science, 1945, p. 63. Shimkin, M. B., and Andervont, H. B.: ibid., p. 97.

those facts and theories which relate to cancer as a phenomenon of abnormal development. All those [540] who have discussed in recent years the problem of cancer from the point of view of developmental mechanics have justly emphasized the obvious fact that cancer cells are not subject to those regulatory mechanisms which normally control growth and differentiation and assure that each part keeps within the limits of the structural pattern of the entire organism. There are indications that in some cases cancerous growth is initiated when a tissue is removed from the regulatory influences of its surroundings. This interpretation might be used in the cases in which carcinoma originates in repeatedly transplanted mammary glands [541] or in those in which explanted and reimplanted cells show malignant growth.[542] Lack of proper regulation may also be claimed for cancer developing in regenerating tissue—for example, in cirrhotic livers, or after subtotal orchiectomy in birds.[543]

In the great majority of instances, however, there is no evidence that a lack of organic control is the cause of cancer. It is quite possible, if not probable, that the lack of response to existing control mechanisms is a fundamental property of cancer cells rather than the cause of their cancerous behavior. The possibility that this change within the cells may be determined by genes, has already been mentioned.

Not in all cases of cancer is there a complete lack of response to normal controls. A well studied example of the effectiveness of such control and its therapeutic application is the carcinoma of the prostate. In many cases the tumor shares with its parent tissue the susceptibility to hormonal influences and regresses when the level of testicular hormone is lowered—for example, by orchiectomy.[544]

The function of cancer cells is well preserved in many cases. These cells may elaborate secretions (e.g., mucus, bile) or hormones, or undergo cornification, or produce a characteristic ground substance (e.g., bone). The function may be much better developed than the structural differentiation would make one believe; this has been observed in hormone-producing cancers.

The resemblance of cancerous and embryonic cells is superficial. It consists in rapid multiplication and a low degree of structural differentiation. On the other hand, one of the fundamental properties, namely, the lack of organization, distinguishes cancer very definitely

540. Waddington, C. H.: Nature, London 135:606, 1935. Needham.[4] Berrill.[537b]

541. Fischer, A.: Am. J. Cancer 31:1, 1937.

542. Earle, W. R., in Research Conference on Cancer, American Association for the Advancement of Science, 1945, p. 139.

543. Champy, C., and Lavedon, J. P.: Compt. rend. Acad. d. sc. 207:99, 1936.

544. Huggins, C.; Stevens, R. E., and Hodges, C. V.: Arch. Surg. 43:209, 1941.

from embryonic tissue, which shows the effect of organizing influences to a high degree.   In cancer, both the .rapid growth and the poor differentiation are related to the lack of control.   The poorly differentiated cancer cell is too abnormal to differentiate properly; the embryonic cell, on the other hand, is normal and may have several possible ways of differentiation depending on its environment.   The cancer cell therefore ·differs more from the embryonic cell with its multiple potencies for differentiation than from the adult cell which may have undergone a reduction of these potencies and is less accessible to organizing influences.   Therefore, a poorly differentiated cancer cell should be called anaplastic and not embryonic.[545]

The just mentioned resemblance of embryonic and cancerous cells accounts for the great interest which many workers have taken in· the rare cases of cancer of the fetus.   The rarity alone is significant, and it illustrates the statement that actually embryonic and cancerous cells are not closely related.   After careful scrutiny, Wells [546] reviews and discusses a limited number of unquestionable cases of prenatal cancer. Among these are no typical cases of carcinoma.   A more recent report of bilateral ovarian carcinoma occurring in a premature infant [547] must be discarded; it deals with normal ovaries in which some of the sex· cords are not yet divided into follicles.   These cords and their transitions into follicles were described as carcinoma cords arising from follicles.   I have seen many similar ovaries in newborn infants.

In conclusion, the meager information concerning the fundamental developmental aspects of cancerous growth can be summarized as follows: Developmental abnormalities, particularly the lack of integration into the pattern of the organism and the poor structural differentiation, are properties rather than causes of cancer.   As Berrill [537b] points out, the incompleteness of present knowledge of the fundamental mechanisms "is due to the standards of reference also being problems as challenging as malignancy itself."   Among the possible causes of cancer which include all types of agents that may produce malformations, two are now being investigated as more universally active than others: somatic mutation and virus infection.

There is one fundamental difference between cancer and most of the other developmental abnormalities which have been discussed in this review.   In the latter, the teratogenic factors act during a short period of development and produce certain changes.   Subsequently, development proceeds by normal mechanisms, and the result is abnormal only because the substrate of these mechanisms is abnormal, owing to

545. Ewing, J.:   Neoplastic Diseases: A Treatise on Tumors, Philadelphia, W. B. Saunders Company, 1941. Gruenwald.[452]
546. Wells, H. G.: Arch. Path. 30:535, 1940.
547. Ziegler, E. E.: Arch. Path. 40:279, 1945.

earlier changes. In cancer, on the other hand, the abnormal mechanism is perpetually active; it has been mentioned that this is one of the facts suggesting a genetic change in the cancer cells by somatic mutation.

CLOSING COMMENT

The present review is based on excerpts from a huge volume of pertinent information scattered throughout the literature of all divisions of medicine, as well as zoology, genetics, veterinary medicine, agricultural research and other fields. In the past, research in developmental pathology has been conducted from many different points of view, and few workers in the field have had a knowledge of previous work in other parts of this discipline, and of some of the basic problems involved. Developmental pathology deserves to be recognized as a portion of biology and pathology in which much has already been accomplished. If further work is to be successful, and if it is to be done in a rational manner, it is necessary to take stock now and endeavor to correlate whatever information is at hand. The present review is an attempt in this direction.

It is obvious that all those disciplines which have contributed to developmental pathology will in turn benefit from the progress made in this field. This holds particularly for practical medicine. Only a few years ago the knowledge of abnormal development had little of practical value to offer to the physician. This changed when within a few years great advances were made in such subjects as erythroblastosis fetalis, cystic fibrosis of the pancreas, malformations caused by rubella during pregnancy and the surgical treatment of several previously hopeless malformations. A stage has been reached in which developmental pathology is bound to make valuable contributions to practical medicine.

Obituaries

GEORGE HOWITT WEAVER, M.D.
1866-1947

George Howitt Weaver was born Oct. 22, 1866, in Waukesha County, Wisconsin. He died at Wilmette, Ill., on April 19, 1947. Dr. Weaver was a graduate of Carroll College, Waukesha; he was of the class of 1889 at Rush Medical College. On completing an eighteen months' internship in Cook County Hospital he engaged in general practice with Dr. Charles Warrington Earle. After Dr. Earle's early death he continued practice for a few years. In the early 90's he spent several months in the study of pathology and bacteriology at the Johns Hopkins Hospital, later carrying on work of the same character with Dr. Hektoen in the John McCormick Institute for Infectious Diseases. From 1905 to 1914 he was attending physician at Cook County Hospital, where he had charge of wards for infectious diseases. In Rush Medical College he was a member of the department of pathology from the time it was organized in 1894, and conducted the early laboratory courses in bacteriology. In 1918 he was appointed professor. In 1902 he joined the staff of the John McCormick Institute for Infectious Diseases and from 1913 to 1933 he served as physician in charge of the Durand Hospital of this institute.

Dr. Weaver was a member of many medical societies: American Medical Association, Illinois State Medical Society, Chicago Medical Society, Chicago Pathological Society, American Association of Pathologists and Bacteriologists, Association of American Physicians and the Institute of Medicine of Chicago. He was the efficient secretary of the Chicago Pathological Society for twenty-five years and also the first secretary of the Society of Medical History of Chicago.

From 1890 to 1933 he made frequent contributions to medical journals, some 65 articles being listed in his bibliography. The earlier articles were reports of cases, their clinical course, diagnosis and pathology. Later articles were especially concerned with the bacteriologic and immunologic aspects of disease and with experimental investigations. He made important pioneer clinical studies on serum disease. Especially valuable were his "Reports of the Durand Hospital of the John McCormick Institute for Infectious Diseases." In these reports, which were made at five year intervals, he embodied not alone much worth while statistical information and results of a scientific study of cases, but dwelt on practical details: the need of a period of observation

before admission of the child to the ward and meticulous efforts to avoid mixed infections; the advantage of the face mask; the extreme importance of careful nursing.

Dr. Weaver was a bibliophile in the true sense, a keen judge of the historic value of a book, a pamphlet or an old college catalogue. His papers on biographic and historical topics are of great interest. He

GEORGE   HOWITT   WEAVER,   M.D.
1866-1947

possessed many items concerned with medical biography and the early history of medical education in Illinois and the West. Many of these items were purchased by Vanderbilt University. A large collection made on behalf of the Society of Medical History of Chicago has gone to the John Crerar Library.

Dr. Weaver was quiet and unassuming. He was of a scholarly bent of mind, activated by a spirit of investigation. In judgment he was well poised; in expression of opinion, clear and accurate. He possessed intellectual as well as ethical honesty. He was fond of flowers and travel; he enjoyed intercourse with congenial friends, to whom he revealed a hitherto unsuspected delicious sense of humor. For several years a distressing physical ailment which he bore uncomplainingly kept him from active participation in medical affairs.

In June 1901 he married Carrie Earle, who survives him.

George H. Weaver will be enrolled as a benefactor of the Institute of Medicine of Chicago, for he willed to it a fund for the endowment of a Charles Warrington Earle Lectureship.

JAMES B. HERRICK.

Books Received

COLLOID SCIENCE: A SYMPOSIUM. Contributors: E. K. Rideal, A. E. Alexander, D. D. Eley, P. Johnson, F. Eirich, R. F. Tucket, J. H. Schulman, M. P. Perutz, G. S. Adair, G. B. B. M. Sutherland and R. R. Smith. Pp. 208, illustrated. Price $6. Brooklyn: Chemical Publishing Company, 1947.

This small book is a collection of condensed elementary lectures, each terminated by a few well selected authoritative references. The lectures deal principally with special aspects of thermodynamics, physical chemistry and organic chemistry as these are used in the development of theory and experiment in colloid science. Emphasis is placed especially on disperse systems, macromolecules and interfacial films composed of one or more layers of molecules. Several important and interesting phases of colloid science are mentioned only briefly or not at all. In general, the book may be regarded as worth while only for those who are wholly unacquainted with the theory, the scope and the methods of colloid science or, more particularly, of chemistry and physics as these are applied to the development of knowledge of the structure and the behavior of disperse systems. Those who are interested in colloid science as applied to biologic systems might find great satisfaction in reading the chapter concerned with the manner in which fat is absorbed from the intestinal lumen.

L'HYPERINSULINIE: LES ÉTATS DE SURACTIVITÉ FONCTIONNELLE DU PANCRÉAS ENDOCRINE EN MÉDECINE EXPÉRIMENTALE ET EN CLINIQUE. By Marcel Sendrail, professeur de pathologie genérale à l'Université de Toulouse. Pp. 256, illustrated. Price 500 francs. Paris: Masson et Cie, 1947.

This monograph presents a well documented review of the various states in which hyperactivity of the endocrine portion of the pancreas is involved. The subject is divided into seven sections under the headings "Biological Concept of Hyperinsulinism," "Experimental Hyperinsulinism," "Clinical Aspects," "Diagnostic Methods," "Causes of Spontaneous Hyperinsulinism," "Extrainsular Hyperinsulinism" and "Therapeutic Problems."

Because of its abundant reference material, especially on the clinical aspects, the book is a valuable one for those clinicians and physiologists who are particularly interested in metabolic and endocrine problems. It contains, however, points of view and deductions from data which are open to much criticism. Often one case report serves the author for a generalization. The discussion of the nervous control of the secretion of insulin is given more prominence than the available knowledge justifies. Hypoglycemia is frequently equated with hyperinsulinism without evidence that the islets are hyperactive.

Despite these critical strictures, the monograph remains a valuable source book.

CALCIFIC DISEASE OF THE AORTIC VALVE. By Howard T. Karsner, M.D., and Simon Koletsky, M.D., Institute of Pathology, Western Reserve University, and the University Hospitals of Cleveland. Pp. 107, with 24 illustrations. Price $5. Philadelphia: J. B. Lippincott Company, 1947.

Based on a thorough analysis of previous studies of calcific disease of the aortic valves and on a careful study of 200 cases observed by the authors, much valid and instructive information is presented in this monograph. There are many excellent photographs, as well as informative tables and charts. Terse and lucid, it is a classic addition to the literature of calcific disease.

INDEX TO VOLUME 44